

EVALUATION OF FISH AND INVERTEBRATE ASSEMBLAGES  
ASSOCIATED WITH TORPEDOGRASS (*Panicum repens*) IN LAKE  
CONROE, MONTGOMERY COUNTY, TEXAS

A Thesis

by

CHRISTOPHER MATTHEW MYNATT

Submitted to the Office of Graduate and Professional Studies of  
Texas A&M University  
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	Frances P. Gelwick
Committee Members,	Michael Masser
	John Oswald
Head of Department,	Michael Masser

December 2016

Major Subject: Wildlife and Fisheries Sciences

Copyright 2016 Christopher Matthew Mynatt

## ABSTRACT

Torpedograss (*Panicum repens*) is a non-indigenous perennial species of rhizomatous graminaceous grass that currently persists along the majority of the vegetated shoreline of Lake Conroe, Montgomery County, Texas. For this study, invertebrate and fish assemblages associated with varying densities of torpedograss were studied seasonally from fall 2015 through summer 2016. Fish assemblages were sampled through the use of exhaustive electrofishing within blocknetted areas containing torpedograss. Invertebrates were sampled using a drop sampler, which was used to collect standardized samples of invertebrates from the water column, torpedograss vegetation, and benthos. Diet composition of piscivorous and insectivorous fishes were also observed.

All fish species collected that comprised at least 2% of the total number of fishes, with exception to largemouth bass, were low dissolved oxygen tolerant species. Kolmogorov-Smirnov tests for length frequency for bluegill (*Lepomis macrochirus*), largemouth bass (*Microperus salmoides*), and western mosquitofish (*Gambusia affinis*) showed no significant difference in length frequency distributions across torpedograss densities; however, significant differences were observed for largemouth bass and western mosquitofish based on seasonality. With regards to invertebrates, a canonical correspondence analysis showed that torpedograss density and weight had the strongest positive correlation (pseudo-F = 25.9, p-value = 0.002) to taxa composition and densities, representing 43.6% of explained variation. Season and location of taxa within

the water column each had a significant positive correlation to the number and diversity of invertebrate taxa and represented 15.8% and 42.4% of explained variation respectively (pseudo-F = 13.0, p-value = 0.002), (pseudo-F = 49.0, p-value = 0.002). The most common invertebrate taxa found within fish diets were also the most common taxa found within torpedograss patches, and chironomid larvae consistently had the highest frequency of occurrence and prey-specific abundance in bluegill, largemouth bass, and golden topminnows (*Fundulus chrysotus*). With regards to fishes, a redundancy analysis showed that torpedograss density and weight had the strongest positive correlation (pseudo-F = 5.1, p-value = 0.002) to fish taxa composition and densities, representing 61.8% of explained variation. Season combined with torpedograss density had a significant positive correlation to the number and diversity of invertebrate taxa and represented 28.4% of explained variation respectively (pseudo-F = 1.7, p-value = 0.09).

Stable isotope analysis supports the use of torpedograss as a foraging location, as the  $\delta^{13}\text{C}$  (‰) values for the largemouth bass, bluegill, and western mosquitofish were all similar, and correlated to the  $\delta^{13}\text{C}$  (‰) value of the invertebrates and periphyton tested. The  $\delta^{13}\text{C}$  (‰) value of torpedograss is relatively close to that of the western mosquitofish, suggesting that the western mosquitofish at some point in its life could be consuming torpedograss itself. These findings provide insight into the fish and invertebrate communities that are utilizing torpedograss as habitat and a potential foraging location. As such, management, rather than eradication, of torpedograss may be preferable when making fisheries management decisions.

## ACKNOWLEDGEMENTS

I would like to give my sincerest thanks to my committee chair, Dr. Gelwick, and my committee members, Dr. Masser and Dr. Oswald for their constant support and approval of this manuscript. Without your continued assistance I would not have been able to come close to being where I am today.

I would also like to thank my friends Mark Webb, Alice Best, Bill Johnson, Mike Gore, and the rest of the crew at the Texas Parks & Wildlife Inland Fisheries office in Snook, Texas. Mark and Alice, you are both amazing examples of what fisheries biologists should be. I thank you both for the advice and amazingly fun, and quite often random, talks we've had. Bill and Mike, the practical knowledge, skillset, guidance, and laughs you've given me has truly shaped the way I view fisheries management. Thanks so much to you both!

I also want to extend a sincere thanks to my friend and fellow Master's student Ryan O'Hanlon for his assistance in field collection and workup. He's put in quite a few man-hours on my project and definitely needs to be acknowledged for his hard work. Thanks a ton!

I would also give my most sincere thanks to my fiancée Victoria Golden who has helped me not only in the field and lab, but also through continuous encouragement and support throughout the entirety of the project. Her help has been amazing.

Special thanks goes to the faculty and staff of the Texas A&M Department of Wildlife and Fisheries for their support in coordinating and managing this project. Their help in handling this project has been wonderful. Thank you all.

## NOMENCLATURE

BG	Bluegill Sunfish
LMB	Largemouth Bass
BW	Bloodworm
AMPH	Amphipod
SJRA	San Jacinto River Authority
TL	Total Length
SL	Standard Length
TPWD	Texas Parks and Wildlife Department
HA	Hectare
RDA	Redundancy Analysis
CCA	Canonical Correlation Analysis

## TABLE OF CONTENTS

	Page
ABSTRACT .....	ii
ACKNOWLEDGEMENTS .....	iv
NOMENCLATURE.....	v
TABLE OF CONTENTS .....	vi
LIST OF FIGURES.....	vii
LIST OF TABLES .....	ix
 CHAPTER	
I        INTRODUCTION .....	1
II        MATERIALS AND METHODS .....	5
Study Setting.....	5
Study Sites .....	6
Field and Laboratory Data Collection.....	10
Data Analysis .....	14
III       RESULTS .....	22
Stomach Content Analysis .....	22
Fish Species Abundance and Length Frequency Distribution.....	24
Multivariate Analysis.....	32
Stable Isotope Analysis.....	42
IV       DISCUSSION AND CONCLUSION .....	44
Fish Assemblage .....	44
Length Frequency Distributions .....	45
Multivariate Fish Analysis.....	46
Multivariate Invertebrate Analysis.....	47
Stomach Content Analysis .....	48
Stable Isotope Analysis.....	49
REFERENCES.....	51

## LIST OF FIGURES

FIGURE	Page
1 Outline of Lake Conroe, Texas, with the Caney Creek Arm outlined in red.....	7
2 Outlined location of Montgomery County in the state of Texas .....	8
3 Study Sampling Sites within the Caney Creek Arm of Lake Conroe, Texas.....	9
4 The explanatory diagram for the Costello 1990 method.....	15
5 Explanatory diagram (center) from Amundsen et al. 1996 for interpretation of feeding strategy, niche width contribution and prey importance from the proposed method, together with characteristic niche utilization curves .....	16
6 Example diagram showing relationships among explanatory groups of variables represented by circles .....	18
7 Plots of mean frequency of occurrence versus mean prey-specific abundance for identified prey in bluegill stomachs (summed across all sampling seasons).....	23
8 Plots of mean frequency of occurrence versus mean prey-specific abundance for identified prey in largemouth bass stomachs (summed across all sampling seasons).....	23
9 Plots of mean frequency of occurrence versus mean prey-specific abundance for identified prey in golden topminnow stomachs (summed across all sampling seasons).....	24
10 Length-frequency histogram of bluegill centimeter groups captured by electrofishing in sparse (s), medium (m), and bare substrate (c) torpedograss vegetation densities.....	25
11 Length-frequency histogram of bluegill centimeter groups captured by electrofishing in fall and winter.....	26
12 Length-frequency histogram of largemouth bass centimeter groups captured by electrofishing in sparse (s), medium (m), and dense (d)	

torpedograss vegetation densities .....	27
13 Length-frequency histogram of largemouth bass centimeter groups captured by electrofishing in spring, summer and winter .....	28
14 Length-frequency histogram of western mosquitofish centimeter groups captured by electrofishing in sparse (s), medium (m), dense (d), and bare substrate (c) torpedograss vegetation densities .....	30
15 Length-frequency histogram of western mosquitofish centimeter groups captured by electrofishing in fall, winter, spring, and summer .....	31
16 Ordination biplot for the RDA of invertebrate assemblage data collected within torpedograss sampling sites in Lake Conroe, Texas .....	36
17 Ordination biplot for the CCA of fish assemblage data collected by electrofishing within torpedograss sampling sites in Lake Conroe, Texas .....	41
18 Mean $\delta^{13}\text{C}$ (‰) vs. mean $\delta^{15}\text{N}$ (‰) ( $\pm 1$ SE) for the primary taxonomic groups from within torpedograss during summer 2016 in Lake Conroe, Texas .....	43



## LIST OF TABLES

TABLE	Page
1 Bluegill sunfish length frequency distribution two-way K/S test D statistic and P-value between density category combinations or between season combinations .....	25
2 Largemouth bass length frequency distribution two-way K/S test D statistic and P-value between density category combinations and season combinations.....	27
3 Western Mosquitofish length frequency distribution two-way K/S test D statistic and P-value between density category combinations and season combinations .....	29
4 Invertebrate taxa identified in Lake Conroe sampling sites during 2015 and 2016 .....	33
5 Significance tests table representing invertebrate variation partitioning with a RDA.....	34
6 Variation explained table representing invertebrate partitioning with a RDA.....	35
7 Fish taxa identified in Lake Conroe sampling sites during 2015 and 2016 .....	38
8 Fish variation partitioning significance tests.....	39
9 Variation explained table representing fish assemblage partitioning with a CCA.....	40
10 Mean $\delta^{13}\text{C}$ (‰) and $\delta^{15}\text{N}$ (‰), as well as C & N standard error values for the primary taxonomic groups from within torpedograss during summer 2016 in Lake Conroe, Texas .....	42

## CHAPTER I

### INTRODUCTION

Submergent and emergent aquatic plants can affect fish abundance and fish distribution by creating structurally complex habitats (Crowder and Cooper 1979) that can greatly affect the composition of fish assemblages. This is in part because vegetation provides fish both cover and foraging locations at multiple stages in their life history (Crowder & Cooper 1982; Conrow et al. 1990). Structurally complex habitats are often positively correlated with invertebrate species abundance, and can influence predator-prey interactions (Dibble et al. 1996; Keast 1984). However, non-native plant species may alter these trends by reducing biotic diversity, altering habitat structure, nutrient cycling, productivity, food web composition, and trophic level dynamics (Zelder & Kercher 2004; Pearson 2009).

Intermediate complexity of aquatic macrophyte structure is most beneficial to bluegill (*Lepomis macrochirus*) and largemouth bass (*Micropterus salmoides*) because food production via epiphytic invertebrates is increased and predation pressure on invertebrates from fishes is decreased (Crowder and Cooper 1982, Durocher et al. 1984, Hoyer and Canfield 1996).

Vegetation-dwelling invertebrates are an important source of food for juvenile and adult fishes, especially in water bodies with lower densities of benthic prey. This is partly due to the strong influence of habitat structure and water quality on invertebrate species composition (Merritt & Cummins 1996). Additionally, morphological variation among aquatic plant species can create structural variety that could influence fish habitat use

(Dibble et al. 1996) interspecific competition (Bettoli & Morris 1991), and affect both individual fish growth and overall population size (Wege and Anderson 1979; Durocher et al. 1984).

Diet studies are used to evaluate food-web dynamics, competition, and predator-prey interactions. Stable isotope analysis offers a different approach to understanding diet patterns by quantifying assimilation of prey over much longer periods than traditional gut content analysis (Pinnegar & Polunin 1999). The phrase “you are what you eat” tends to apply particularly well to stable isotope analysis, as it may be fairly representative of an organism’s composition when observing carbon and nitrogen isotope values of an organism. Stable-carbon isotope ratios ( $^{13}\text{C}/^{12}\text{C}$ ) of tissues represent the relative contributions of carbon sources that are consumed (DeNiro & Epstein 1978), while stable-nitrogen isotope ratios ( $^{15}\text{N}/^{14}\text{N}$ ) represent the relative contributions of prey trophic levels, allowing estimation of a predator’s trophic position (Post 2002).

Torpedograss (*Panicum repens*) is a perennial species of rhizomatous graminaceous grass in the tribe Paniceae, subfamily Panicoideae, and family Poaceae (Sutton 1996). In the continental United States, the genus *Panicum* contains more than thirty species. Of those, approximately 25 are native to the United States and seven have been introduced into the United States from other parts of the world (Freckmann & LeLong 2006). Torpedograss, which is a non-indigenous invasive species, was initially introduced into the United States in the late 19<sup>th</sup> century as forage for agricultural species such as cattle (Tarver 1979). It inhabits wetlands and other naturally aquatic areas in multiple tropical and temperate regions of the world (Sutton 1996) including Australia (Holm et al. 1977), Europe (Tarver 1979), and Africa (Waterhouse 1994). Torpedograss has very long-lived rhizomes, and can grow

under conditions ranging from soils low in moisture to soils under several meters of water.

While torpedograss can spread via seed, it has an extremely low germination rate (0.1%) in the southern United States (Sutton, 1996). Unfortunately, torpedograss often forms high stem density stands and thickly entangled mats that prevent usage by larger fishes. This increased density causes torpedograss to often be considered to be undesirable habitat, especially for game fishes and wading birds (Hanlon and Langeland 2000). However, the density torpedograss exhibits has been shown to correlate with increased densities of invertebrates (Warren & Hohlt 1994).

Torpedograss is extremely prevalent in Lake Conroe, and persists along much of the vegetated shoreline. Entities, such as Texas Parks and Wildlife, have historically made attempts to establish native species of vegetation in the littoral zone of Lake Conroe following introduction of a strongly herbivorous fish species, grass carp (*Ctenopharyngodon idella*), to the lake. However, due to the invasive nature of torpedograss (Holm et al. 1977), it is often difficult for native vegetation to fully establish along the shores of Lake Conroe. Management efforts are not currently being taken to reduce the overall biomass of torpedograss within Lake Conroe. Because the ecological attributes of torpedograss within Lake Conroe are unknown, this study will focus on fish and invertebrate assemblages associated with torpedograss in Lake Conroe to aid in future fisheries management decisions.

Primary objectives of this research were to:

1. Document and compare composition of fish and invertebrate assemblages found within different densities of torpedograss in Lake Conroe in each of four consecutive seasons from fall 2015 through summer 2016;
2. Document and compare the gut contents and diet composition of common piscivorous and insectivorous fish species captured across vegetation densities in each of four consecutive seasons from fall 2015 through summer 2016;
3. Sample taxa from multiple trophic levels that represent littoral torpedograss communities during summer 2016 and document ( $^{15}\text{N}/^{14}\text{N}$ ) & ( $^{13}\text{C}/^{12}\text{C}$ ) stable isotope ratios to elucidate nutrient pathways between trophic levels.

## CHAPTER II

### MATERIALS AND METHODS

#### Study Setting

Lake Conroe is an 8,500 hectare (~20,000 ac) reservoir (Figure 1), impounded in 1973, located on the West Fork of the San Jacinto River in Walker and Montgomery counties, Texas. The reservoir, which is approximately 65 kilometers north of Houston, Texas (Figure 2), is utilized for water storage for Houston. It is managed and operated by the San Jacinto Water Authority and has a typically stable water level. The Sam Houston National Forest encompasses the northern half of the reservoir, allowing for much of the northern half consist of vegetated shoreline. However, the southern half of the reservoir is very highly developed with residential housing and businesses and consists primarily of bulk-head and rip-rap, with very few instances of vegetated shoreline. Thus developed and vegetated shoreline habitats create a distinct contrast between the two halves of the lake. Species of emergent macrophytes that populate the vegetated shorelines of Lake Conroe, include *Panicum repens* (torpedograss), *Panicum hemitomon* (maidencaine), *Justicia americana* (water willow), *Hydrocotyle spp.* (water pennywort), *Scirpus californicus* (bulrush), *Pontederia cordata* (pickerelweed), and *Nymphaea odorata* (white water lily). However, torpedograss dominates the majority of the vegetated shoreline of Lake Conroe (M. Webb, personal communication, July 2015).

## Study Sites

Beginning in October 2015, various torpedograss sites were sampled for fishes and invertebrates within the Caney Creek arm of Lake Conroe, Montgomery County, Texas (Figure 3) in four consecutive seasons starting in autumn 2015. All sampling occurred during daylight hours. Prior to each sampling season, a comprehensive vegetation survey of the Caney Creek arm of lake Conroe was completed. The purpose of the vegetation survey was to identify all occurrences of pure torpedograss in patches of at least 5m x 1m sampling areas. Patches containing were tagged (GPS), and designated as one of the following four categories based on their percentage of plant cover within the sampling area:

1. Bare Substrate - No vegetation present
2. Sparse: 1-30% torpedograss coverage
3. Medium: 31-70% torpedograss coverage
4. Dense: 71-100% torpedograss coverage

Five sampling sites were randomly chosen within each coverage category in each season, resulting in a total of 80 sites sampled. Random samples were considered more appropriate than repeated sampling of the same sites because observations indicated that coverage at a site is highly variable over time due to changes in water level that allow grass carp (*Ctenopharyngodon idella*) access to graze on littoral vegetation.

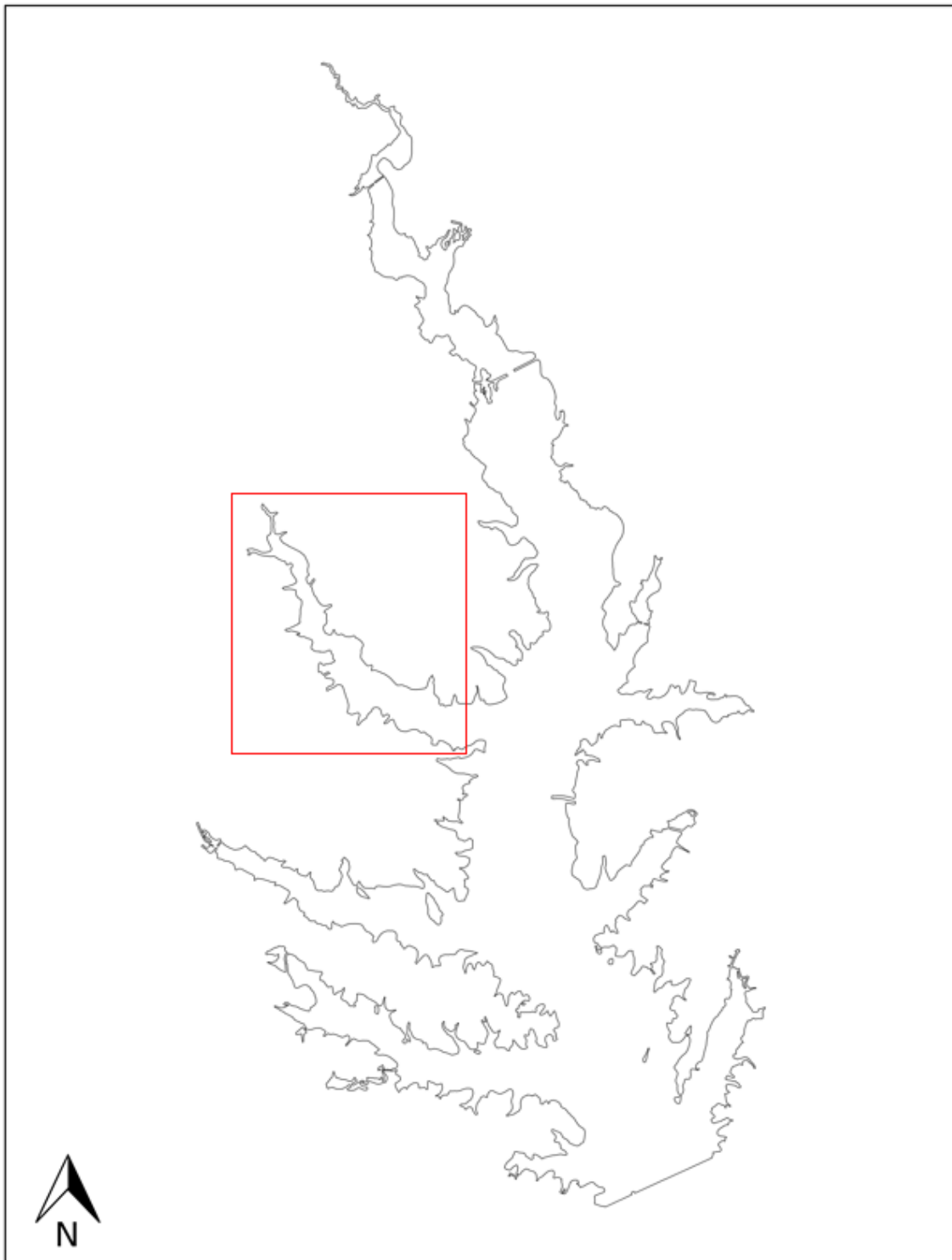


Figure 1. Outline of Lake Conroe, Texas, with the Caney Creek Arm outlined in red.





Figure 2. Outlined location of Montgomery County in the state of Texas.

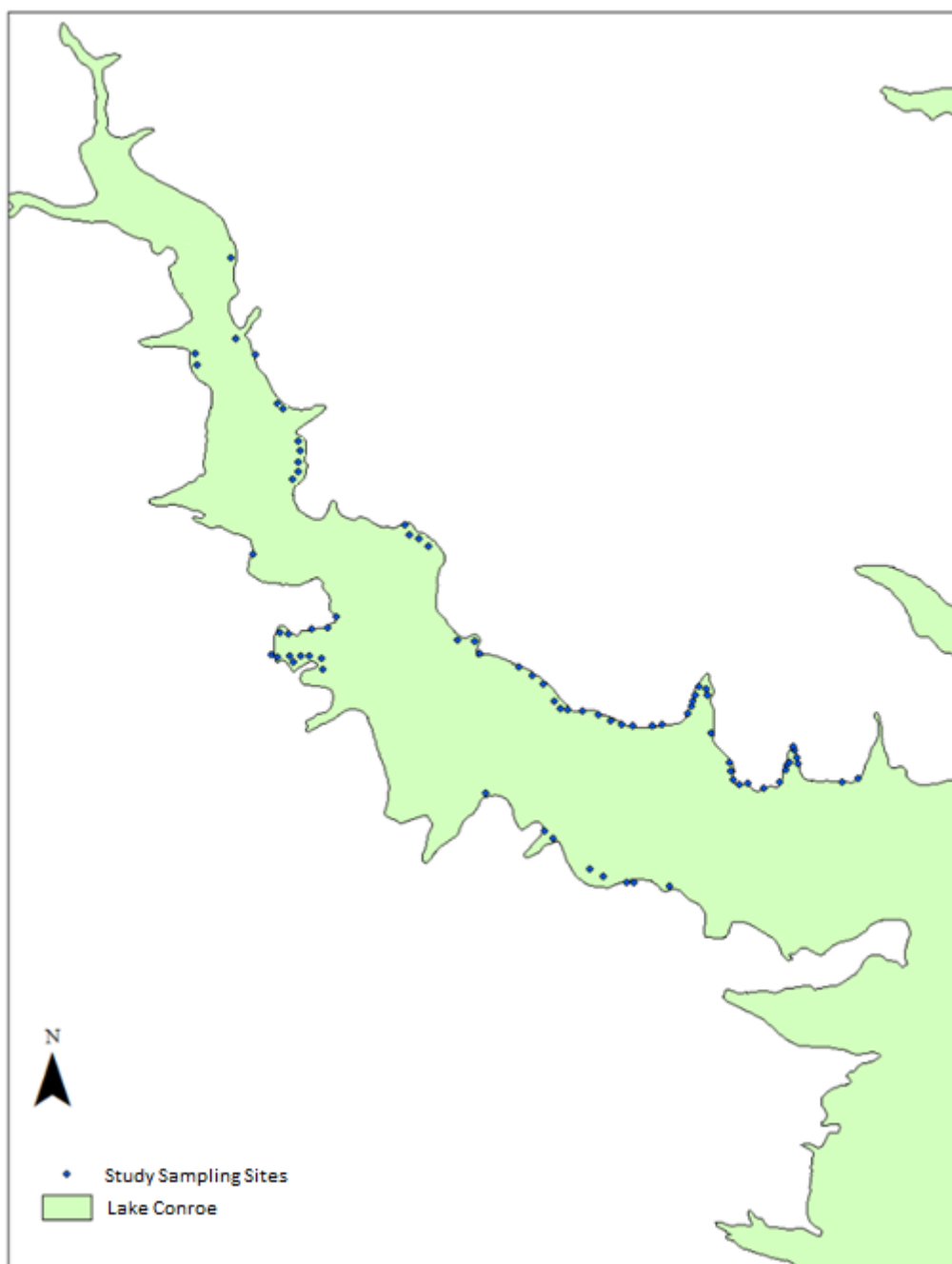


Figure 3. Study Sampling Sites within the Caney Creek Arm of Lake Conroe, Texas.

## **Field and Laboratory Data Collection**

Fishes were sampled by electrofishing, which is a preferred method within the shallow littoral zone (Reynolds 1996). Invertebrates from within each of the benthos, water column, and vegetation areas were sampled using a drop sampler, an effective method when sampling epiphytic habitats (Merritt et al. 1984, Turner 1997).

### *Vertebrate Sampling Protocol*

Sampling effort at each site was standardized by utilizing an 18.29m x 0.91m (60ft x 6ft) blocknet (0.64-cm diameter hexagonal mesh) to surround a rectangular 1m x 5m area. The blocknet was deployed by starting at the shoreline, then slowly walking the net to a point one meter from shore, and dropping the blocknet into the water, extending the net 5 meters parallel to shore, and then returning to shore. The shoreline side of the sampling rectangle had no net, and ends were anchored to the shoreline using lead weights. The rectangular shape of the net was maintained by using three 2m tall steel conduit as corner posts, and as a middle supporting post, 1m from shore; and, posts were installed after initial net deployment. The following water quality parameters were recorded: water temperature (°C), pH, total dissolved solids (TDS), conductivity (µSiemens), dissolved oxygen (mg/L) and salinity (ppt). Water quality parameters were recorded using an YSI Pro Plus ® after setting the block net, but prior to electroshocking.

The area within the blocknet was then electrofished using a DC-pulsed current via a portable modified Coffelt electrofishing unit powered by a Honda 5000W

generator. The area was exhaustively electrofished, with a minimum electrofishing time of three minutes. One person collected fishes using a dip net (20x20x20-cm net with 3-mm mesh). The dip net was used to repeatedly sweep through and disturb the vegetated sampling area to ensure all stunned fish were collected. Fishes collected from each sampling area were immediately put into plastic bags and placed in a cooler containing ice-water. Each bag was labeled with the date and GPS coordinates of the sampling site. Sampled fish were transported to the Texas Parks & Wildlife Department (TPWD) Inland Fisheries office in Snook, Texas at the end of each sampling day and were stored in a freezer (-4 °C) until they could be further processed.

#### *Invertebrate Sampling Protocol*

After fishes were collected, invertebrates were sampled using a drop sampler (internal diameter of 0.5m, height 0.9m) at one location within the block-netted sample area. The location to be sampled was decided by dividing the 5m x 1m sample area into five 1m x 1m sectors, assigning each sector a reference number. A random number generator was then used to randomly select one sector to be sampled using the drop sampler. The drop sampler was then placed in the randomly selected location and hammered into the substrate.

The depth to which the drop sampler was hammered varied, but always fulfilled two criteria: (1) the drop sampler was buried at least 0.15-m into the sediment, and (2) water did not seep back into the sampler through the sediment after water is pumped out. After the drop sampler was installed, water was removed from within the drop sampler

by using a manually operated diaphragm pump. All water leaving the drop sampler via the diaphragm pump was immediately filtered through a sieve (500- $\mu$ m mesh) and organisms caught by the sieve were placed in a plastic bag, tagged as a water column sample, and immediately placed in a cooler with ice-water. After removing the water column sample, all vegetation at the sediment surface was physically removed from within the drop sampler by clipping stems at sediment level. Special care was taken to minimize disturbance from the sediment surface. All plant material was immediately placed in plastic bags, then placed in ice-water to preserve the sample until it could be processed. Benthic material was then collected using a hand trowel. One gallon (3.79 liter) of soil and benthic material was removed, and measured using a marked and calibrated plastic bucket. Each sample was transferred to a plastic bag, tagged and immediately placed in ice-water until it could be processed.

#### *Lab Protocols for Processing Invertebrate Samples*

Invertebrates found in the water column, vegetation, and benthos were separately picked and stored in order to identify their assemblage composition. Subsamples that had been sieved from the water column were immediately preserved in 85% ethanol and labeled. Vegetation subsamples were taken out of their plastic storage bag, allowing excess water to drain back into the bag, and weighed ( $\pm$  1g) as damp “wet weight”. Invertebrates were picked from the weighed vegetation, labeled, and placed in 85% ethanol. The soil/benthos samples were wet washed through a 500- $\mu$ m mesh sieve. Invertebrates were picked while sieving the benthos, labeled and placed in 85%

ethanol.

Invertebrates from each sample category were sorted, identified to the lowest practical taxonomic level and counted if the total count was less than 200 individuals. If there were more than 200 invertebrate individuals, then the Environmental Protection Agency's Rapid Bioassessment subsampling methods for invertebrates were followed by evenly spreading organisms into a light-colored pan with a numbered grid pattern (Barbour, 1999). Four squares within the grid pattern were randomly chosen using a random number generator and individuals within each square were identified to the lowest practical taxon and counted. Individual organisms within squares were sorted and counted in their entirety until the combined total 200 individual count  $\pm$  20% was reached. If the four squares combined contained greater than 200 individuals, then the contents of the four squares were combined and spread into a second pan identical to the first and processed to sort and count individuals. This methodology was repeated for each of the three invertebrate habitat categories (water column, vegetation, and benthos) within each sample.

#### *Lab Protocols for Processing Fish Samples*

Frozen fish samples from each site were thawed and blotted to remove excess water. Each fish was weighed (g), its total length was measured (mm), and identified to species before its stomach contents were determined. The true stomach (from the esophagus to the anterior portion of the intestine) of all insectivorous and piscivorous species was removed and the contents processed. Individual prey items were identified

to the lowest practical taxonomic level.

## **Data Analysis**

### *Condition (Relative Weight)*

Relative weight (Wr) indices, which may be used to estimate the physiological health of individual fish (Anderson & Neumann, 1996), were not used for this analysis as almost all fishes caught were too small for this analysis.

### *Stomach Content Analysis*

The stomach content data was used to calculate frequency of occurrence for each prey taxon in each fish species, which is represented by the equation:

$$O_i = J_i / P$$

$O_i$  is the frequency of occurrence of the prey taxon across conspecific fish in a particular sample,  $J_i$  is the number of conspecific fish that consumed an identified prey taxon ( $i$ ), and  $P$  is the number of conspecific fish that contained food. The values for this metric range from 0 to 1 (indicating occurrence from rare to prevalent). Frequency of occurrence gives an indication of the homogeneity of prey items in a fish species' diet, as well as indicate how common a prey item is in a diet, but cannot assess the prey's overall importance to the fish species.

Stomach content data was also used to calculate prey-specific abundance, which is a graphical technique that depicts prey abundance as a function of its frequency of

occurrence (Amundsen et al. 1996). Prey-specific abundance is a modification of the model by Costello (1990) (Figure 4) in which prey-specific abundance ( $P_i$ ) is defined as the percentage a prey taxon comprises of all prey items in only those predators in which the actual prey occurs (Amundsen et al. 1996). It is represented by the equation:

$$P_i = (\sum S_i / \sum S_{ti}) \times 100$$

$P_i$  is the prey-specific abundance of prey  $i$ ,  $S_i$  is the number of prey  $I$  across all stomachs, and  $S_{ti}$  is the total number of all prey items across all stomachs containing prey  $I$ . A combination of these two diet measures can explain diet variability, relative importance of each prey type, and feeding strategies (Figure 5).

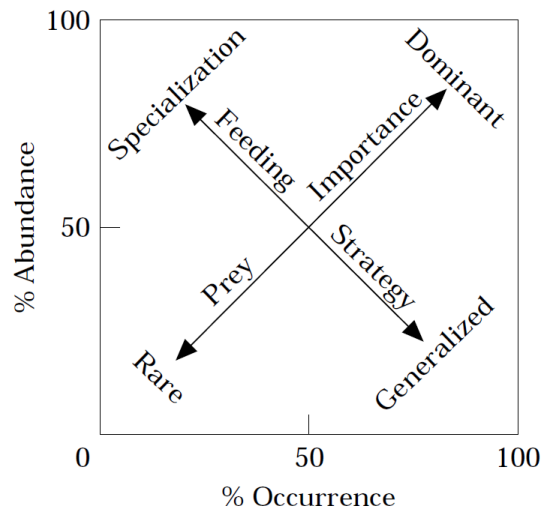


Figure 4. The explanatory diagram for the Costello 1990 method.



# GRAPHICAL ANALYSIS OF FEEDING STRATEGY

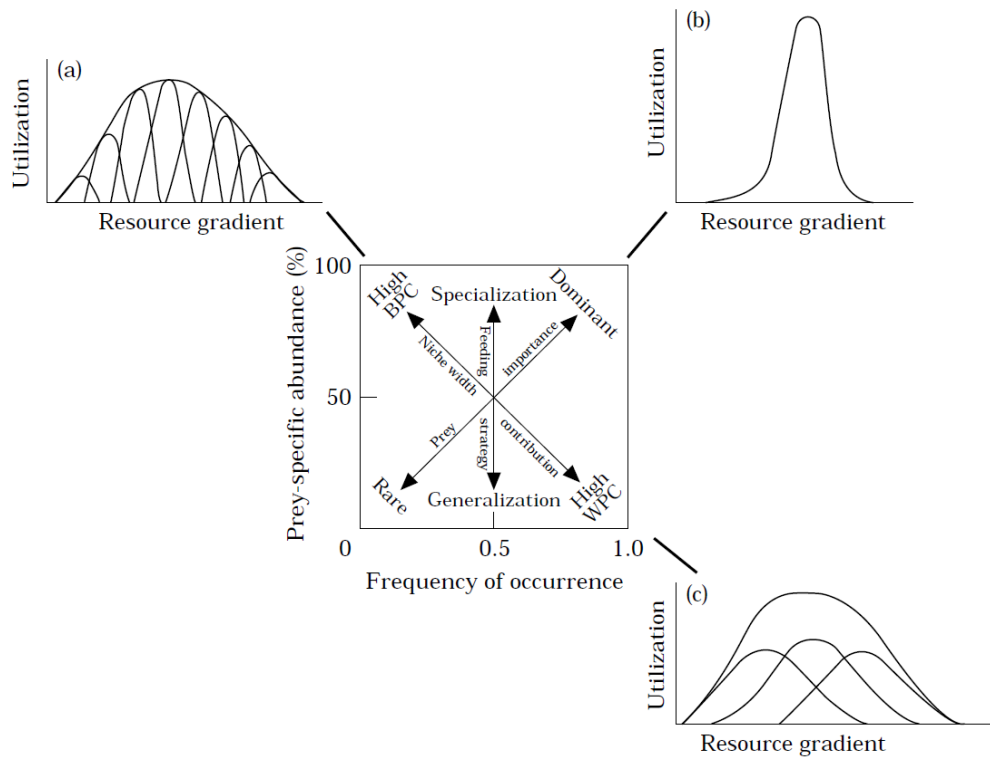


Figure 5. Explanatory diagram (center) from Amundsen et al. 1996 for interpretation of feeding strategy, niche width contribution and prey importance from the proposed method, together with characteristic niche utilization curves. (a) High between-phenotype component to niche width, (b) narrow niche width and (c) high within-phenotype component.

## *Length Frequency and Abundance of Fishes*

The effect of season and torpedograss density categories on length frequency distributions for total lengths (TL) was calculated for fish species. Fish species with fewer than twenty individuals sampled across all four study seasons were excluded from analysis. The TL of individuals for each species were plotted as length frequency distributions using 1.0-cm intervals for all species. Statistical analyses were performed using the SAS 9.4 statistical analysis program. For each fish species, Kolmogorov-

Smirnov (K-S) tests were used to test for differences in length-frequency distributions (with conspecific fish data pooled across all seasons) among density categories of torpedograss, and for seasonal differences in length-frequency distribution (with conspecific fish data pooled across all torpedograss density categories). The K-S test is a popular non-parametric method used to determine differences in length-frequencies, since such data often differ significantly from a normal distribution (Neuman and Allen 2007). The K-S test is sensitive to differences in both location and shape of the data distribution. Furthermore, the K-S test is appropriate for skewed and multi-modal length frequency data, as it makes no underlying assumptions about data distribution (Neuman and Allen 2007). The K-S test calculates the Z-statistic, which is the largest absolute distance between cumulative distribution functions (D), using a significance level of  $P \leq 0.05$ .

### *Multivariate Methods*

Multivariate statistical methods offer an impartial approach to ascertaining patterns in species assemblages and their relationships with environmental conditions (Jackson et al., 2001). Analysis using multivariate methods was conducted using the Canonical Community Ordination software (CANOCO 5.0). Preliminary analyses indicated that linear ordination methods explained a greater amount of the variation in invertebrate data. Therefore, a redundancy analysis (RDA), was applied to invertebrate data to explain the relationships between response (invertebrate taxa) and explanatory variables by constraining the canonical ordination axes to be linear combinations of

explanatory variables (ter Braak and Smilauer, 2002). The explanatory variables used for ordination of invertebrate data were season, vegetation density category (sparse, medium, dense, and bare substrate), vegetation weight (g), and location of sample (benthos, water column, vegetation).

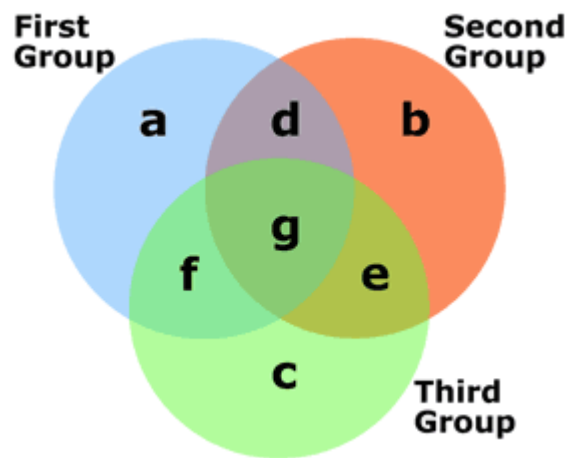


Figure 6: Example diagram showing relationships among explanatory groups of variables represented by circles. Letters correspond to individual estimated fractions of unique and shared variation in the assemblage that is explained by each group. Note that the Venn diagram is not drawn to scale.

Preliminary analyses indicated that unimodal ordination explained a greater amount of the variation in fish data. Therefore, a canonical-correlation analysis (CCA), which is a unimodal constrained multivariate statistical method, was used to evaluate variation within fish assemblages, collected by electrofishing within torpedograss, and infer associations between explanatory variables and fish assemblage structure. The

explanatory variables used in the CCA for fish assemblage data were season, vegetation density (sparse, medium, dense, bare substrate), and vegetation weight (g).

A variation partitioning analysis was conducted for fishes using a CCA, as well as for invertebrates using a RDA, to partition the variation in the response data into parts attributed to sets of explanatory variables (ter Braak and Smilauer, 2002). Three sets of explanatory variables (predictor groups) were tested for their unique and shared effects (Figure 6). The analysis happens over a stepwise selection process, completed for every predictor group independently (ter Braak and Smilauer, 2002). This allows the analysis to base the estimates of explained variation on the less biased percentage of explained variation, calculated in the same way as the adjusted R<sup>2</sup> statistic in multiple regression (ter Braak and Smilauer, 2002). Significance tests for variation explained by each axis and by explanatory variables and variation partitioning were based on Monte Carlo randomizations.

#### *Methods for Stable Isotope Analysis*

All samples for stable isotope analysis were collected during the summer of 2016 within the Caney Creek arm of Lake Conroe, Montgomery County, Texas from areas of torpedograss. The fish and invertebrate taxa collected for stable isotope analysis were chosen based on their high prevalence within torpedograss. The fishes included juvenile (<120 mm TL) largemouth bass (*Micropterus salmoides*), bluegill (*Lepomis macrochirus*), and western mosquitofish (*Gambusia affinis*). Fishes were collected by electrofishing within the sampling area by using a boat mounted electrofishing unit

powered by a 5000-watt generator, and were immediately placed in plastic bags, labeled and placed into a cooler containing ice water. The entirety of torpedograss sites were sampled to ensure adequate collection of desired fishes for stable isotope analysis.

Invertebrates were collected from the water column, torpedograss stems and leaves, and benthos as described for previous invertebrate samples. The invertebrate taxa chosen for stable isotope analysis were amphipods (*Hyalella azteca*), chironomid larvae (chironomidae), and ostracods (ostracoda). Multiple individuals from each taxon were homogenized in order to have an adequate (1-mg dry weight) sample of tissue per taxon for stable isotope analysis. Plankton samples were acquired by collecting 20-liter water samples from the Caney Creek arm of Lake Conroe, Montgomery County, Texas, placing the samples in ice-water, and transporting them to the lab at Texas Parks & Wildlife Inland Fisheries office in Snook, Texas. Ten samples of torpedograss were removed from multiple locations within the Caney Creek arm of Lake Conroe, Montgomery County, Texas.

All samples were transported to the Texas Parks & Wildlife Inland Fisheries office in Snook, Texas at the end of each sampling day. All samples, except those of plankton, periphyton, and torpedograss, were stored in a freezer at -4 °C until they could be further processed.

Plankton was immediately filtered from each water sample through A 1.5-μm Fisher® G6 borosilicate glass fiber filter by utilizing a Gast® Model 0211 vacuum pump. Plankton samples on the filter were dried in an oven at 60 °C for 48 hours, and then ground together into homogenous powder using a Retsch® Oscillating Mixer Mill

(MM400). Inclusion of the borosilicate glass fiber filter does not affect the  $^{13}\text{C}$  or  $^{15}\text{N}$  signatures due to the absence of carbon or nitrogen within the filter. Periphyton for stable isotope analysis was removed from torpedograss by scraping the periphyton from the stems using a blunt probe. The torpedograss was then washed with tap water and brushed to remove any remaining periphyton and debris prior to stable isotope analysis.

Fishes were thawed; and, dorsal muscle tissue was dissected from juvenile largemouth bass for use in the stable isotope analysis (Bodin et al. 2007). Bluegill and western mosquitofish individuals collected for stable isotope analysis did not have sufficient amounts of dorsal muscle tissue for analysis, so the whole fish was used for analysis. Ten individual fish were used as replicate samples of each species. The thawed samples of fish and invertebrates were separately dried in an oven at 60 °C, cut into 1cm segments and ground into homogenous powder using a Retsch Oscillating Mixer Mill (MM400). Once ground, 1 mg ( $\pm 0.02$  mg) of each sample was weighed out into a tin cup, using a microbalance, and sealed for analysis of  $^{15}\text{N}$  and  $^{13}\text{C}$  at the Texas A&M University Stable Isotopes for Biosphere Science Laboratory, College Station, Texas, following standard protocols (Fry, 2006).

## CHAPTER III

### RESULTS

#### **Stomach Content Analysis**

The fish species that had stomach contents available for analysis were bluegill (*Lepomis macrochirus*), largemouth bass (*Micropterus salmoides*), and golden topminnow (*Fundulus chrysotus*). Plant material was not found in the stomachs of any of these fishes. Prey for Bluegill (N=13) included four invertebrate taxa: amphipods, chironomid larvae, backswimmers, and ostracods. Chironomid larvae were the most common and abundant prey for bluegill and were found in all bluegill stomachs ( $O_i = 1$ ,  $P_i = 92.93$ ) (Figure 7). Largemouth bass (N=29) stomachs contained eight invertebrate taxa: amphipods, chironomid larvae, caddisflies, crayfish, damselflies, whirligig beetles, backswimmers, and ostracods. Chironomid larvae were the most common and abundant prey for largemouth bass and were found in most largemouth bass stomachs ( $O_i = 0.83$ ,  $P_i = 49.89$ ) (Figure 8). Crayfish had the highest prey-specific abundance; however, they only occurred in two largemouth bass individuals ( $O_i = 0.03$ ,  $P_i = 50$ ). Golden topminnows (N=2) stomachs contained five invertebrate taxa: amphipods, chironomid larvae, crawling water beetles, backswimmers, and ostracods. As with the bluegill and largemouth bass, the most common and abundant prey of golden topminnows were chironomid larvae, which occurred in all stomachs containing food ( $O_i = 0.03$ ,  $P_i = 50$ ) (Figure 9).

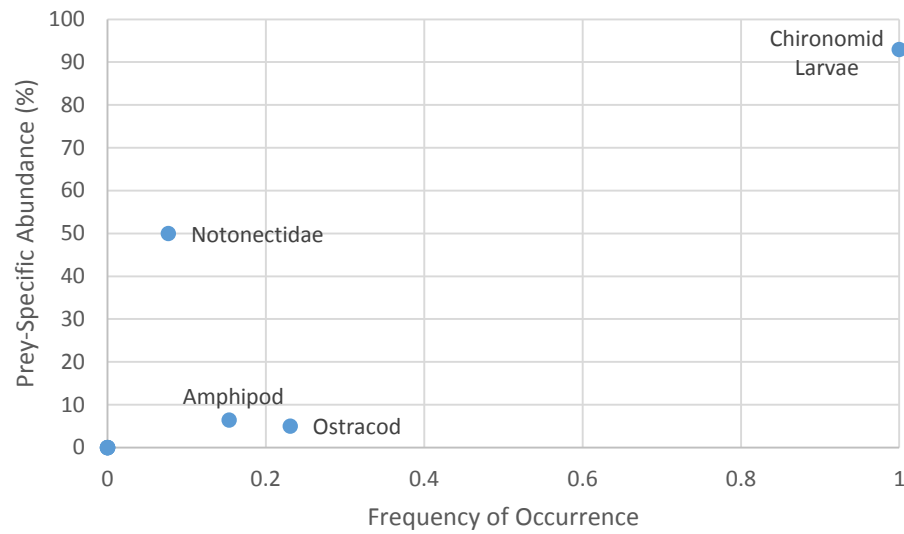


Figure 7. Plots of mean frequency of occurrence versus mean prey-specific abundance for identified prey in bluegill stomachs (summed across all sampling seasons). N = 13 (stomachs with contents). The most common prey types are labeled for comparison.

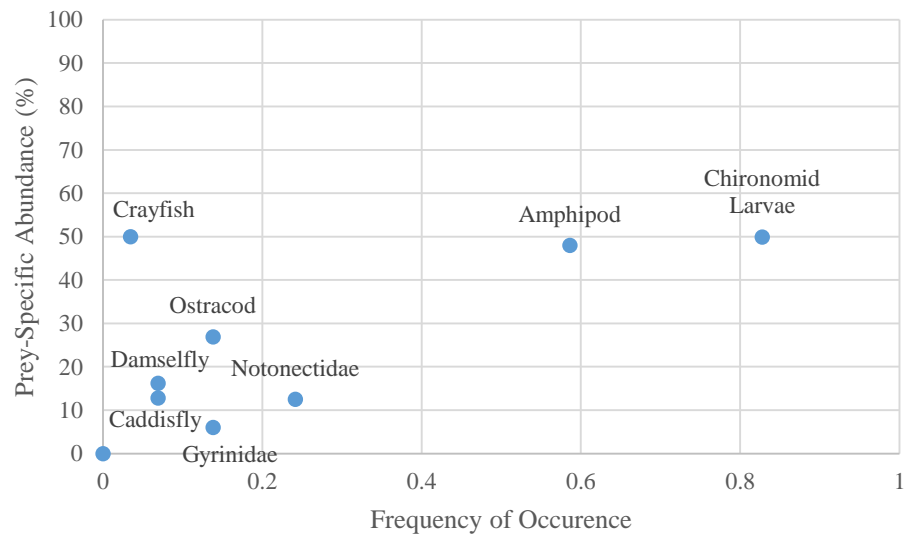


Figure 8. Plots of mean frequency of occurrence versus mean prey-specific abundance for identified prey in largemouth bass stomachs (summed across all sampling seasons). N = 29 (stomachs with contents). The most common prey types are labeled for comparison.



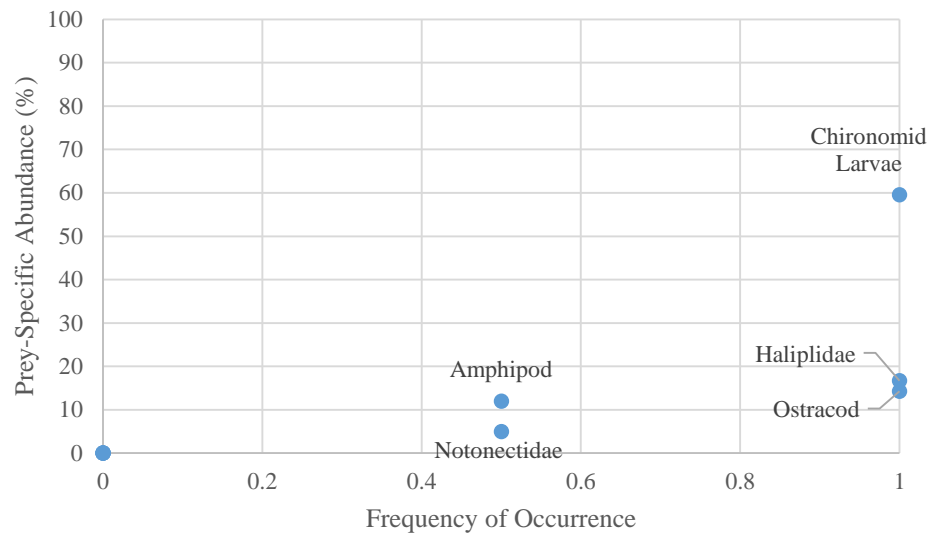


Figure 9. Plots of mean frequency of occurrence versus mean prey-specific abundance for identified prey in golden topminnow stomachs (summed across all sampling seasons). N = 2 (stomachs with contents). The most common prey types are labeled for comparison.

## Fish Species Abundance and Length Frequency Distribution

### *Bluegill*

The number of bluegill captured by electrofishing was highest in medium density sites (n=9), and lowest in dense sites (n=0) (Figure 10). Mean TL for bluegill across all seasons was 32.63 mm (Figure 11) and mean weight was 0.864 g (n=17). The K-S tests for length frequency distributions showed no significant differences in shape or location of the distribution of bluegill amongst torpedograss densities or amongst seasons (Table 1).

Table 1. Bluegill sunfish length frequency distribution two-way K/S test D statistic and P-value between density category combinations or between season combinations. No individuals were found in the Spring, Summer, or dense sites.

	<i>D</i> Value	<i>P</i> Value
Bare/Medium	0.35	0.72
Sparse/Bare	0.12	1.0
Medium/Sparse	0.44	0.48
Fall/Winter	0.28	0.99

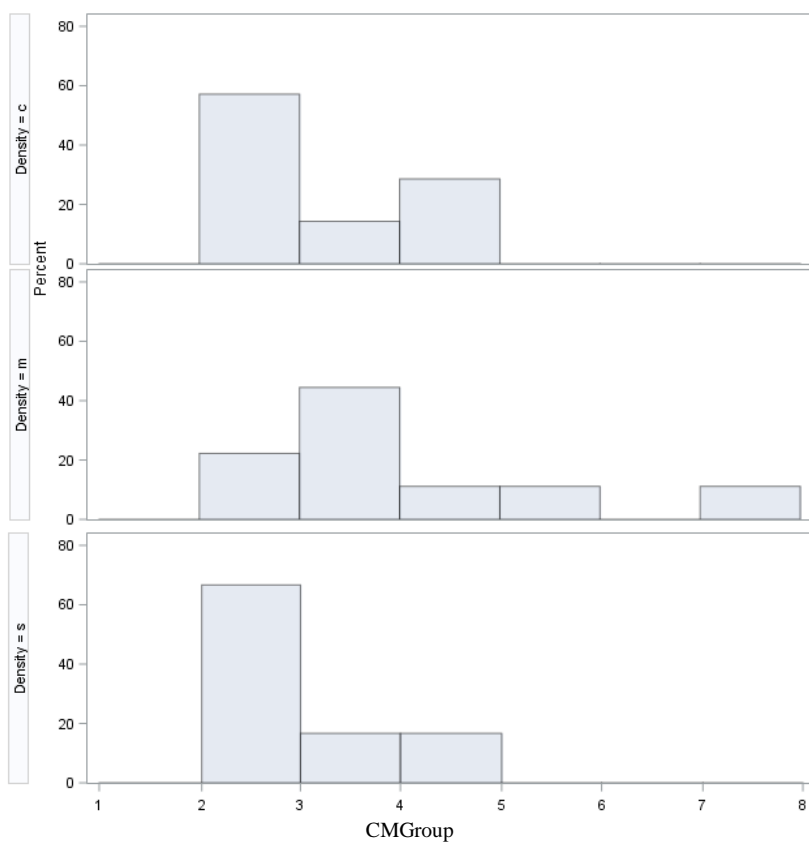


Figure 10. Length-frequency histogram of bluegill centimeter groups captured by electrofishing in sparse (s), medium (m), and bare substrate (c) torpedograss vegetation densities. No bluegill were found in dense sites.

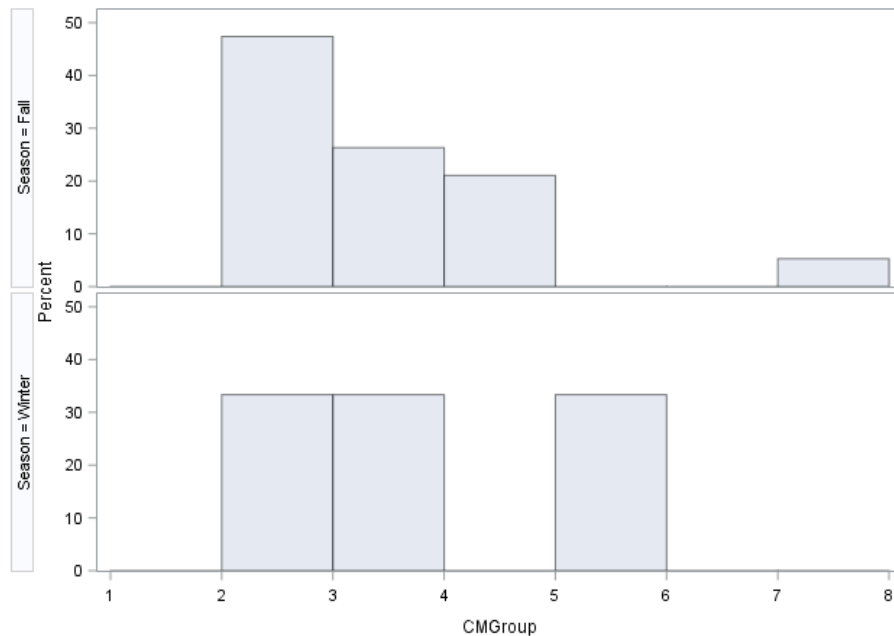


Figure 11. Length-frequency histogram of bluegill centimeter groups captured by electrofishing in fall and winter. No bluegill were found in the spring or summer.

### *Largemouth Bass*

The number of largemouth bass captured by electrofishing was highest in medium density sites (n=19), and lowest in sites with bare substrate (n=0) (Figure 12). Mean total length for largemouth bass across all seasons was 55.09 mm (Figure 13) and mean weight was 3.56 g (n=35). The K-S tests for length frequency distributions showed that there were no significant differences in shape or location of the distribution of largemouth bass amongst torpedograss densities (Table 2). However, there were significant differences among seasons where largemouth bass were observed: between winter and spring ( $D = 1.0$ ,  $P = 0.01$ ), between summer and winter ( $D = 1.0$ ,  $P = 0.02$ ), and between spring and summer ( $D = 0.95$ ,  $P < 0.01$ ) (Table 2).

Table 2. Largemouth bass length frequency distribution two-way K/S test D statistic and P-value between density category combinations and season combinations. No individuals were found in bare substrate sites, or during the Fall.

	<i>D</i> Value	<i>P</i> Value
Dense/Medium	0.38	0.19
Medium/Sparse	0.78	0.21
Sparse/Dense	0.79	0.23
Winter/Spring	1.0	0.01
Winter/Summer	1.0	0.01
Summer/Spring	0.95	<0.01

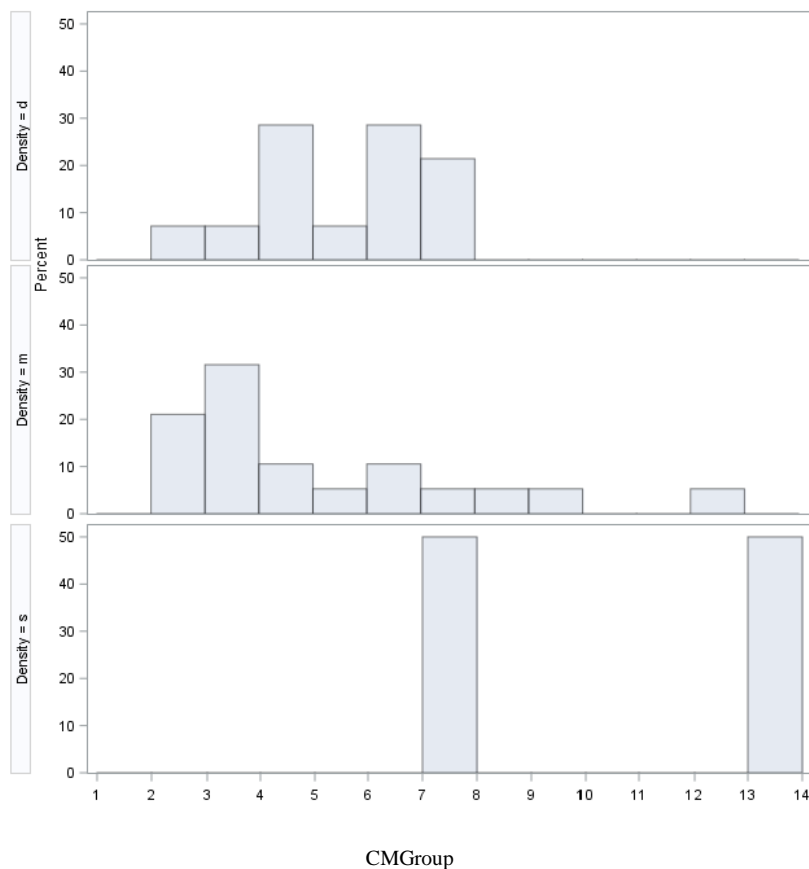


Figure 12. Length-frequency histogram of largemouth bass centimeter groups captured by electrofishing in sparse (s), medium (m), and dense (d) torpedograss vegetation densities. No largemouth bass were found in bare substrate sites.

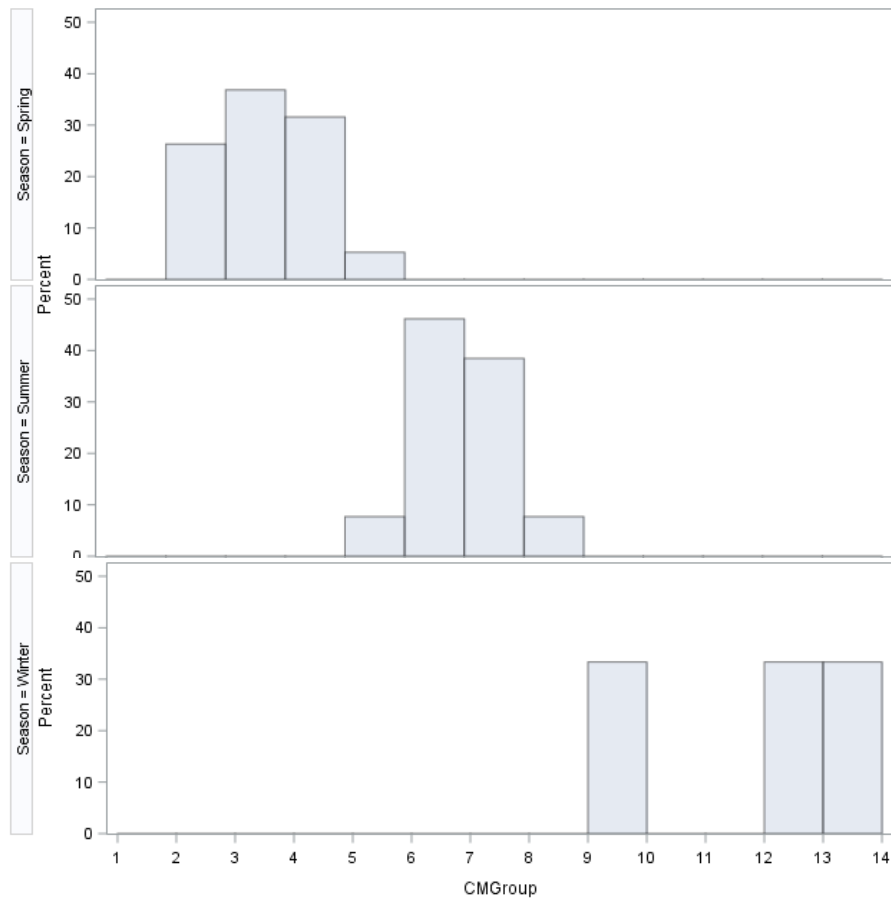


Figure 13. Length-frequency histogram of largemouth bass centimeter groups captured by electrofishing in spring, summer and winter. No largemouth bass were found in the fall.

### *Western Mosquitofish*

The number of western mosquitofish captured by electrofishing was highest in medium density sites (n=30), and lowest in sites with bare substrate (n=1) (Figure 14). Mean total length for western mosquitofish across all seasons was 29.07 mm (Figure 15) and mean weight was 0.62 g (n=73). The K-S tests for length frequency distributions showed that there were no significant differences in shape or location of the distribution of western mosquitofish amongst torpedograss densities (Table 3). However, there were

significant differences among seasons: between fall and summer ( $D = 0.56$ ,  $P < 0.02$ ), and between summer and spring ( $D = 0.45$ ,  $P < 0.03$ ) (Table 3).

---

Table 3. Western Mosquitofish length frequency distribution two-way K/S test  $D$  statistic and  $P$ -value between density category combinations and season combinations.

---

	<i>D</i> Value	<i>P</i> Value
Dense/Medium	0.33	0.08
Bare/Medium	0.67	0.78
Dense/Bare	0.33	0.99
Sparse/Bare	0.20	1.00
Medium/Sparse	0.47	0.03
Sparse/Dense	0.13	0.99
Fall/Winter	0.15	1.00
Fall/Spring	0.11	1.00
Fall/Summer	0.56	0.03
Winter/Spring	0.17	1.00
Winter/Summer	0.53	0.47
Summer/Spring	0.45	0.03

---

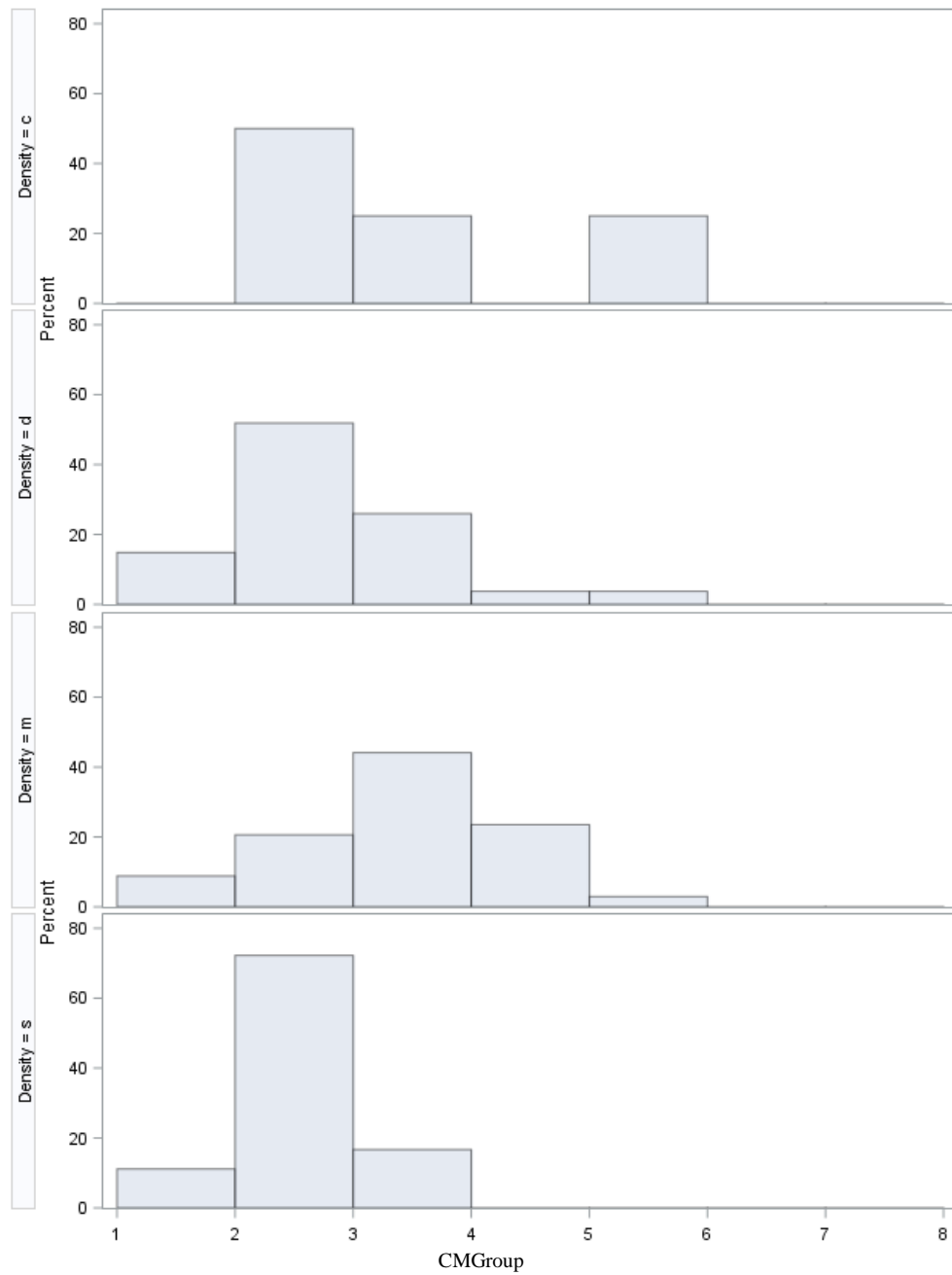


Figure 14. Length-frequency histogram of western mosquitofish centimeter groups captured by electrofishing in sparse (s), medium (m), dense (d), and bare substrate (c) torpedograss vegetation densities.

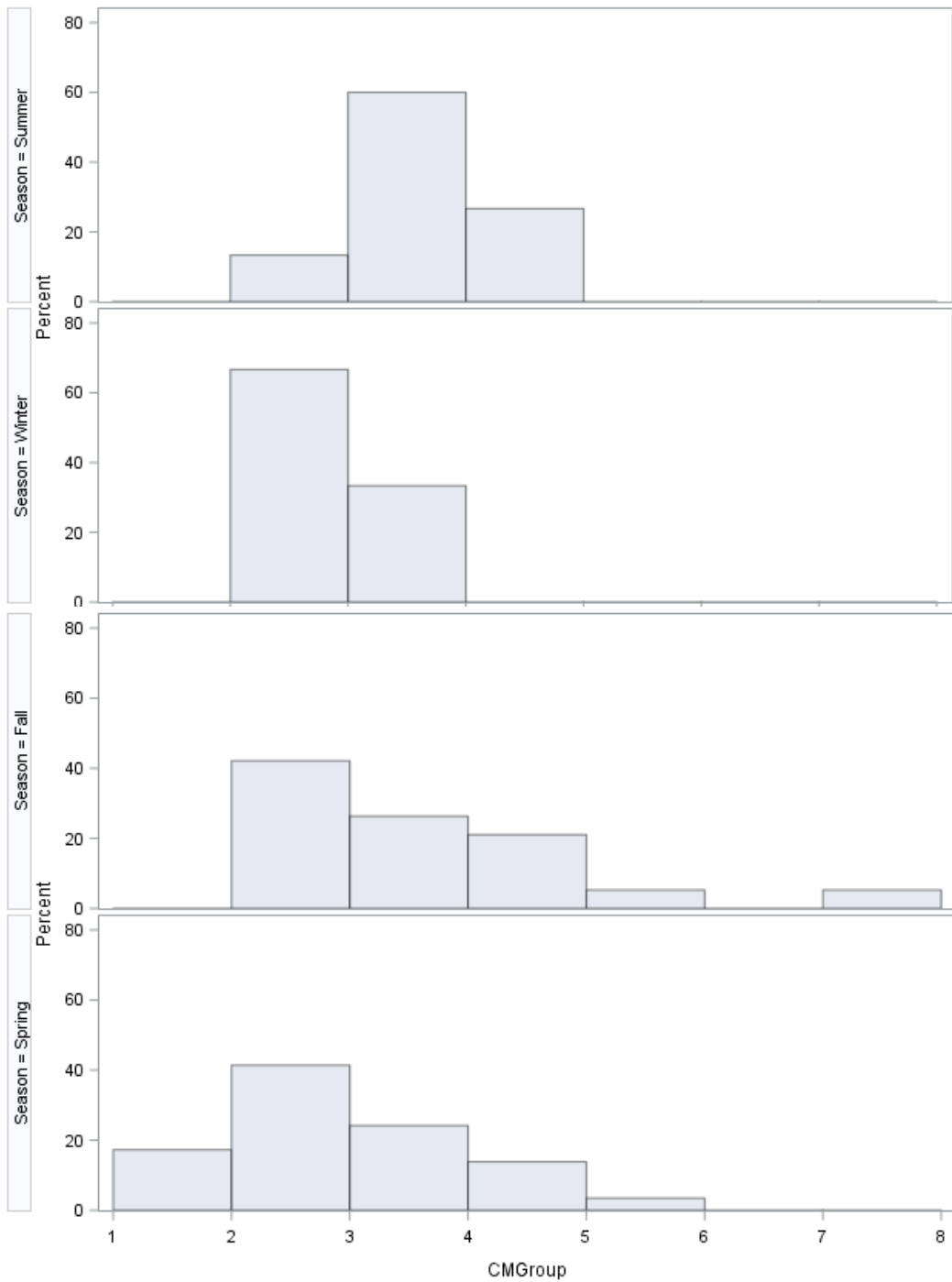


Figure 15. Length-frequency histogram of western mosquitofish centimeter groups captured by electrofishing in fall, winter, spring, and summer.



## Multivariate Analysis

### *Invertebrate Multivariate Analysis*

A total of 38 invertebrate taxa, representing 17 orders were captured in sites over all 2015 and 2016 sampling seasons combined (Table 4). The RDA of invertebrate taxa and explanatory variables showed that the first axis, as well as all canonical axes combined explained a significant amount of variation in the data matrix (Axis 1 = 35.38%, pseudo-F = 126,  $p = 0.002$ ; all canonical axes combined = 51.3%, pseudo-F = 26.9,  $p\text{-value} = 0.02$ ). In order to simplify the biplot (Figure 16) only those invertebrate taxa that cumulatively represent 95% of explained variation within the RDA are shown. A closer proximity of a centroid to a canonical axis indicates its higher correlation with the axis. The biplot shows that the first axis depicts the ecological gradient of invertebrates that was associated with density and weight of torpedograss (Figure 16). Axis two depicts the gradient related to season, and sample location in the water column. Chironomid larvae were strongly correlated with sparse densities of torpedograss, as well as with the winter season (upper left quadrat of Figure 16). All other invertebrate taxa, including damselflies, snails, caddisflies, mayflies, amphipods, water boatmen, backswimmers, and giant water bugs, tended to be much more strongly correlated to increasing vegetation weight, as well as dense and medium categories of torpedograss density. Overall invertebrate taxa tended to be negatively correlated with the bare substrate category, the spring season, and samples taken from the benthos (lower right quadrat of Figure 16).

Table 4. Invertebrate taxa identified in Lake Conroe sampling sites during 2015 and 2016.

<u>Taxa</u>	<u>Common Name</u>	<u>Major Habitat</u>	<u>N</u>	<u>Percent Composition</u>
<i>Hyaella azteca</i>	Amphipods	Aquatic	68606	82.294
Chironomidae	Chironomid larvae	Aquatic	10271	12.320
Cicadellidae	Leafhopper	Terrestrial	2002	2.401
Annelida	Annelid worm	Aquatic or Terrestrial	572	0.686
<i>Physella spp.</i>	Snail	Aquatic	299	0.359
<i>Caenis spp.</i>	Mayfly	Aquatic	228	0.273
Corixidae	Water boatman	Aquatic	181	0.217
Belostomatidae	Giant water bug	Aquatic	158	0.190
Notonectidae	Backswimmer	Aquatic	142	0.171
Trichoptera	Caddisfly	Aquatic	142	0.171
<i>Tetragnatha spp.</i>	Long-jawed orbweaver	Aquatic	161	0.193
Diplura	Two-pronged bristletails	Terrestrial	115	0.138
Hirudinea	Leech	Aquatic	97	0.117
Hydrophilidae	Water Scavenger beetle	Aquatic	62	0.075
Zygoptera	Damselfly	Aquatic	61	0.073
Gyrinidae	Whirligig beetle larvae	Aquatic	43	0.051
Halipidae	Crawling water beetle	Aquatic	36	0.043
Vellidae	Riffle bugs	Aquatic	32	0.038
Dytiscidae	Predaceous diving beetle	Aquatic	27	0.032
Formicidae	Ant	Terrestrial	20	0.024
<i>Procambarus clarkii</i>	Crayfish	Aquatic	16	0.019
<i>Palaemonetes spp.</i>	Common grass shrimp	Aquatic	15	0.018
Libellulidae	Skimmer dragonfly	Aquatic	15	0.018
Carabidae	Ground beetle	Terrestrial	14	0.017
<i>Corbicula spp.</i>	Basket clam	Aquatic	13	0.016
Ephydriidae	Shore fly	Aquatic	9	0.011
Asellidae	Isopod	Aquatic	8	0.009
Tipulidae	Crane fly larvae	Aquatic	5	0.006
Curculionidae	Weevil	Aquatic or Terrestrial	3	0.004
Gomphidae	Clubtail dragonfly larvae	Aquatic	3	0.004
Cleridae	Checkered beetle	Terrestrial	2	0.002
Gryllidae	Cricket	Terrestrial	2	0.002
Ceratopogonidae	Biting midge larvae	Aquatic	1	0.001
	Emerald dragonfly larvae			
Corduliidae	larvae	Aquatic	1	0.001
Gryllotalpidae	Mole cricket	Terrestrial	1	0.001
Hebridae	Velvet water bug	Aquatic	1	0.001
<i>Macrobrachium spp.</i>	Freshwater prawn	Aquatic	1	0.001
Simuliidae	Black fly	Aquatic	1	0.001

The variation partitioning analysis of the invertebrate data showed that all combinations of the first group (season), second group (vegetation weight, categorical stem density), and third group (location in water column) of explanatory variables, as well as unique variation for each group were significant (Table 5). Table 6 summarizes the results of permutation tests made per each analytical step and evaluation of either the effects of fractions for each unique contribution of individual groups, or the effects of combined groups (ter Braak and Smilauer, 2002).

Table 5. Significance tests table representing invertebrate variation partitioning with a RDA. The type I error and pseudo-F statistic are shown in the P and F columns, respectively. For this analysis the first group was season (a), the second group was vegetation weight and stem density (b), and the third group was position in the water column (c). Other letters indicate shared variation between the main groups as depicted in Figure 6.

Tested Fraction	F	P
a+b+c+d+e+f+g	26.9	<0.002
a	13	<0.002
b	25.9	<0.002
c	49	<0.002
a+d	9.5	<0.002
b+e	18.3	<0.002
c+f	42.4	<0.002

Vegetation weight and stem density together (Table 6) uniquely explained the greatest amount of variation (43.6%). Location in the water column and seasonality each uniquely explained the second and third greatest amount of explained variation (42.4% and 15.8% respectively). Explained variation provided by variation from  $a \cap b = d$ ,  $b \cap c = e$ ,  $a \cap c = f$ ,  $a \cap b \cap c = g$  was relatively small comparatively, accounting for -1.99% of the total explained variation.

Table 6. Variation explained table representing invertebrate partitioning with a RDA. The *% of explained* column shows the percentage of the variation that can be explained by the explanatory variables in the response data. The *% of all* shows the total contribution of the individual fractions and are expressed as percentages of the total amount of variation (*Variation (adj)*) found in the data. *DF* represents the degrees of freedom, and *Mean Square* shows the explained variation divided by the degrees of freedom, which approximately corresponds to the mean squares from the analysis of variance.

Fraction	Variation(adj)	% of Explained	% of All	DF	Mean Square
a	0.078189	15.8	7.8	3	0.02753
b	0.21521	43.6	21.5	4	0.0548
c	0.20942	42.4	20.9	2	0.10376
d	-0.00074498	-0.2	-0.1	--	--
e	-0.0053733	-1.1	-0.5	--	--
f	-0.0033389	-0.7	-0.3	--	--
g	0.00012956	0.01	0.01	--	--
Total					
Explained	0.49349	100	49.3	9	0.05695
All Variation	1	--	100	239	--

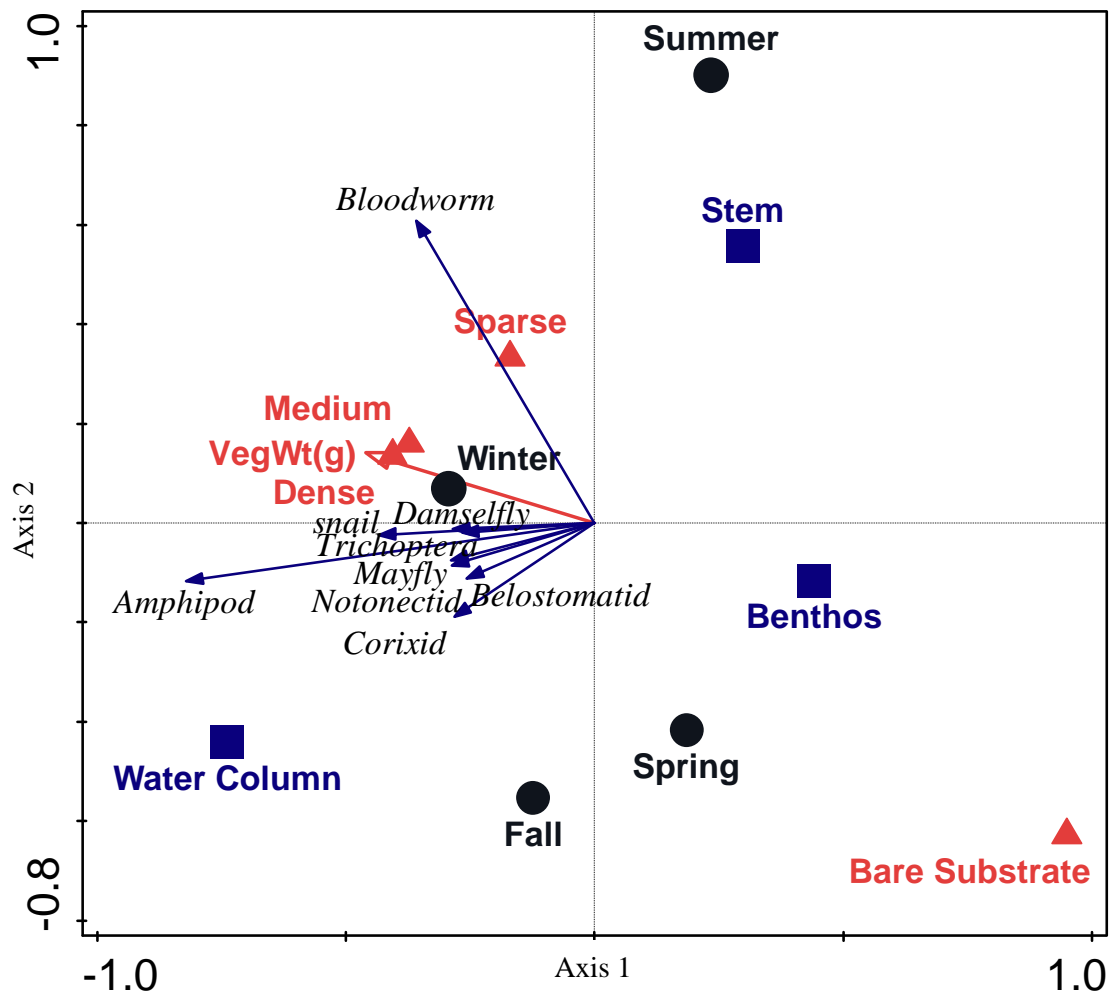


Figure 16. Ordination biplot for the RDA of invertebrate assemblage data collected within torpedograss sampling sites in Lake Conroe, Texas. Relationships are depicted between vegetation weight (red arrow), season (black circle), categorical torpedograss density (blue square), and invertebrate taxa (blue arrow). Centroids for invertebrate taxa are plotted according to their correlation with the canonical axes.

### *Fish Multivariate Analysis*

A total of 11 fish species, representing seven families were captured using electrofishing over all samples (Table 7). Six species comprised greater than 2% of the total number of fishes are hereafter referred to as 'common fishes'. A CCA was conducted analyzing the distribution and abundance of common fish species amongst sampling sites, along with the explanatory variables associated with each site (Figure 17). The first axis of the CCA, as well as all axes combined, were significant (Axis 1 = 20.66%,  $p = 0.004$ , pseudo-F value = 7.0; all canonical axes combined = 35.0%,  $p$ -value = 0.012, pseudo-F value = 2.1, adjusted explained variation = 6.3%). Within the CCA, the first axis depicts the ecological gradient associated with the density and weight of torpedograss. Axis two depicts the gradient related to season. The CCA taxa and environmental variables biplot shown in Figure 17 contains all common fishes that cumulatively represent 100% of explained variation (35.0% of all variation). Closer proximity of a centroid indicates its higher correlation with the canonical axis. Western mosquitofish were correlated with dense torpedograss, and negatively correlated with bare substrate sites. In contrast, bullhead minnows were strongly correlated with bare substrate sites, and negatively correlated to dense categories of torpedograss. Largemouth bass were strongly correlated to the spring and summer seasons, as well as to medium densities of torpedograss. Bluegill sunfish and inland silversides tended to be negatively correlated to largemouth bass, summer, spring, and medium densities of torpedograss; and, they were positively correlated with fall, winter, and sparse categories of torpedograss (Figure 17).

---

Table 7. Fish taxa identified in Lake Conroe sampling sites during 2015 and 2016.

---

**Common Fish Taxa**

<b>Family</b>	<b>Scientific Name</b>	<b>Common Name</b>	<b>N</b>	<b>Percent Composition</b>
Poeciliidae	<i>G. affinis</i>	Western mosquitofish	117	56.25
Centrarchidae	<i>M. salmoides</i>	Largemouth bass	34	16.35
Centrarchidae	<i>L. macrochirus</i>	Bluegill sunfish	22	10.58
Atherinidae	<i>M. beryllina</i>	Inland silverside	11	5.29
Cyprinidae	<i>P. vigilax</i>	Bullhead minnow	7	3.37
Fundulidae	<i>F. chrysotus</i>	Golden topminnow	5	2.40

**Uncommon Fish Taxa**

<b>Family</b>	<b>Scientific Name</b>	<b>Common Name</b>	<b>N</b>	<b>Percent Composition</b>
Cyprinidae	<i>N. texanus</i>	Weed shiner	4	1.92
Ictaluridae	<i>A. natalis</i>	Yellow bullhead	3	1.44
Cyprinidae	<i>C. venusta</i>	Blacktail shiner	2	0.96
Ictaluridae	<i>N. nocturnus</i>	Freckled madtom	2	0.96
Aphredoderidae	<i>A. sayanus</i>	Pirate perch	1	0.48

---

The variation partitioning analysis showed that only certain combinations of groups were significant. The combinations shown to be significant were ( $a + b + d + e, + f + g$ ) ( $p = 0.012$ ),  $b + e$  ( $p = 0.002$ ), and  $c + f$  ( $p = 0.048$ ) Table 8 (ter Braak and Smilauer, 2002).

Table 8. Fish variation partitioning significance tests. The type I error and pseudo-F statistic are shown in the P and F columns, respectively. For this analysis the first group was season (a), the second group was vegetation weight (b), and the third group was stem density (c). Other letters indicate shared variation between the main groups as depicted in Figure 6.

Tested Fraction	F	P
a+b+c+d+e+f+g	2.1	0.012
a	1.5	0.138
b	0.6	0.592
c	1.2	0.272
a+d	1.7	0.09
b+e	5.1	0.002
c+f	2	0.048

Fraction  $e$ , the unique explained variation by stem density (fraction  $b$ ) and the variation it shared with vegetation weight (fraction  $c$ ), explained the greatest amount of variation in fish distribution (68.7% of explained variation; Table 9). The variation uniquely explained by seasonality and that shared with vegetation weight ( $a \cap c = f$ ) accounted for the second greatest percentage of explained variation (30.7%). The unique component explained by season (fraction  $a$ ) and the unique component explained by stem density explained the third and fourth greatest amount of explained variation (22.8% and 10.5% respectively).



Table 9. Variation explained table representing fish assemblage partitioning with a CCA. summarize the results of permutation tests made per each analytical step and evaluation of either the effects of fractions for each unique contribution of individual groups, or the effects of multiple fractions that have been combined together

Fraction	Variation(adj)	% of Explained	% of All	DF	Mean Square
a	0.10142	22.8	4.1	3	0.08906
b	-0.03051	-6.9	-1.2	1	0.03411
c	0.046918	10.5	1.9	3	0.07303
d	0.024991	5.6	1	--	--
e	0.30605	68.7	12.4	--	--
f	0.13681	30.7	5.6	--	--
g	-0.14044	-31.5	-5.7	--	--
Total					
Explained	0.44524	100	18.1	7	0.12284
All					
Variation	2.4592	--	100	34	--

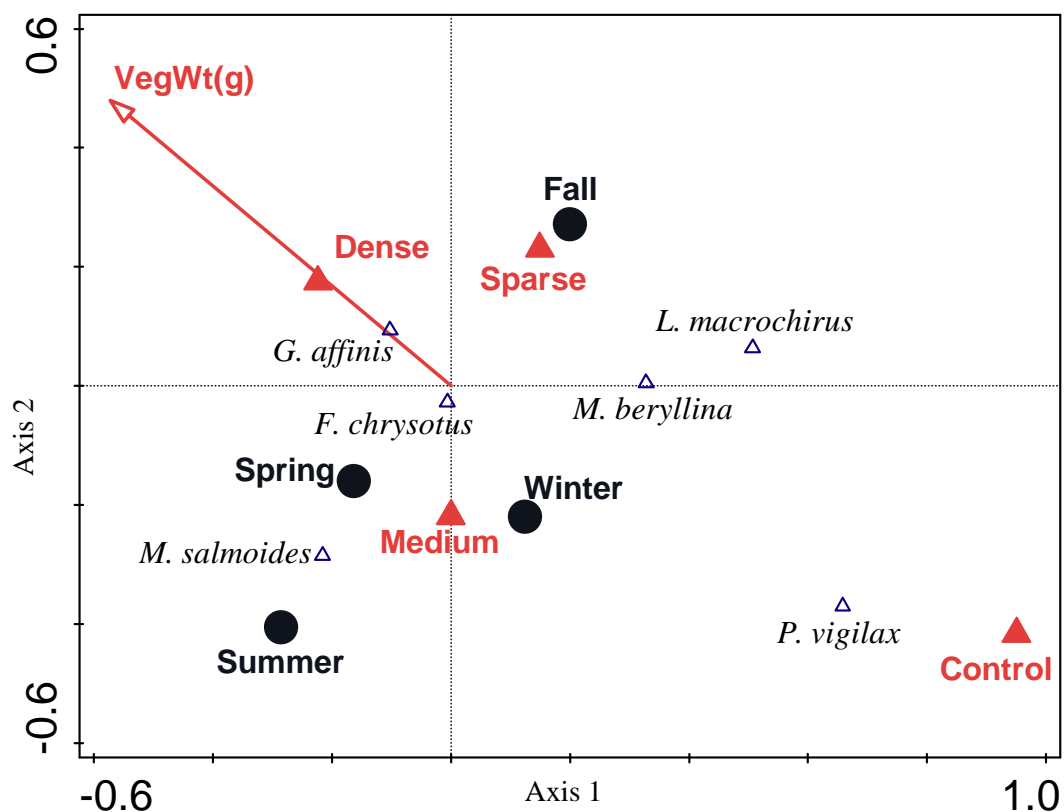


Figure 17. Ordination biplot for the CCA of fish assemblage data collected by electrofishing within torpedograss sampling sites in Lake Conroe, Texas. Relationships are depicted between vegetation weight (red arrow), season (black circle), categorical torpedograss density (red triangle), and fish species (open triangle). Centroids for fish species are plotted according to their correlation with the canonical axes.

## Stable Isotope Analysis

Stable isotope analysis showed bluegill and largemouth bass to be closely related in both  $\delta^{15}\text{N}$  (‰) and  $\delta^{13}\text{C}$  (‰) values (Table 10), whereas western mosquitofish were closely correlated to  $\delta^{13}\text{C}$  (‰) values but had lower  $\delta^{15}\text{N}$  (‰) values. Chironomid larvae, amphipods, and ostracods had relatively similar  $\delta^{15}\text{N}$  (‰) values, but were spread over a large range with regards to  $\delta^{13}\text{C}$  (‰) (Figure 18).

Table 10. Mean  $\delta^{13}\text{C}$  (‰) and  $\delta^{15}\text{N}$  (‰), as well as C & N standard error values for the primary taxonomic groups from within torpedograss during summer 2016 in Lake Conroe, Texas

Taxa	Mean $\delta^{13}\text{C}$ (‰)	Mean $\delta^{15}\text{N}$ (‰)	Std. error N	Std. error C
Torpedograss	-13.27	6.91	0.076430797	0.126260313
LMB	-26.46	11.93	0.233458062	0.420105278
Bluegill	-27.40	12.45	0.234866629	0.308407378
Mosquito fish	-25.36	8.45	0.320814562	0.46144491
Bloodworm	-28.69	3.07	--	--
Amphipod	-22.37	2.90	--	--
Ostracod	-19.35	2.65	--	--
Periphyton	-25.40	3.56	--	--
Plankton	-26.80	1.99	--	--

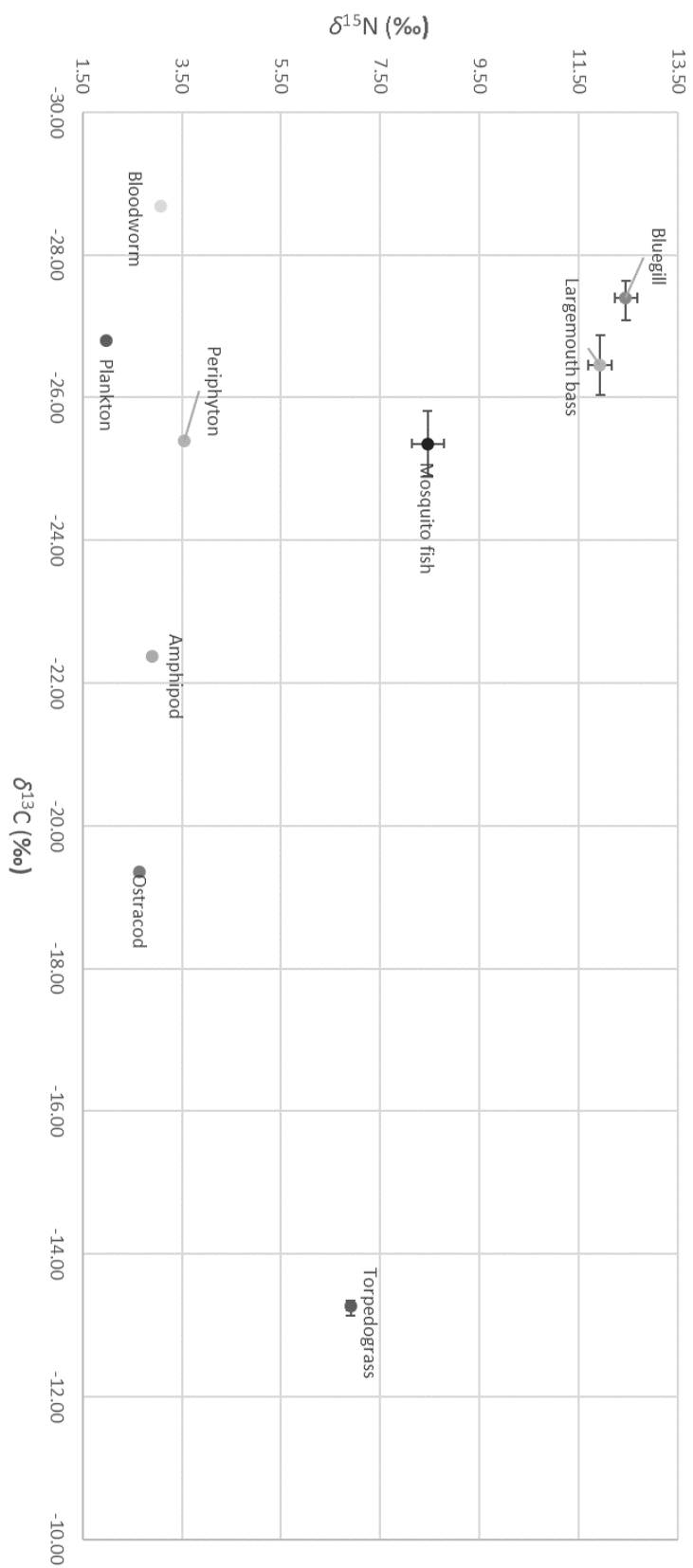


Figure 18. Mean  $\delta^{13}\text{C}$  (‰) vs. mean  $\delta^{15}\text{N}$  (‰) ( $\pm 1$  SE) for the primary taxonomic groups from within torpedograss during summer 2016 in Lake Conroe, Texas

## CHAPTER IV

### DISCUSSION AND CONCLUSION

The results from this evaluation provided several implications with regards to how the invertebrate and fish assemblages can be affected torpedograss within Lake Conroe, Texas.

#### **Fish Assemblage**

Warren and Hohlt (1994) described torpedograss dominated habitats as characterized by epiphytic invertebrate and fish communities that tolerate low dissolved oxygen. This is in part due to the dense growth form of torpedograss that often reduces the amount of open water available for fish to move through, and reduces light levels for periphyton growth (Havens & Gawlik 2005). These general trends were also observed in torpedograss sites sampled in Lake Conroe. The majority of fishes collected (69.23%, N = 144) were physiologically tolerant species such as western mosquitofish (56.25%, N = 117), bluegill (10.58%, N = 22), and golden topminnow (2.40%, N = 5). Interestingly largemouth bass, which normally persist in normoxic water (Linam et al. 2002), were the second most prevalent species (N = 34), comprising 16.35% of total fishes collected from torpedograss sites. These fish were all less than 120-mm TL, indicating that largemouth bass may be using torpedograss as nursery habitat during their smaller, more invertivorous stage, whereas larger, more piscivorous, individuals may not usually inhabit stands of torpedograss. This is because larger-bodied fishes are more likely to

have difficulty maneuvering through the dense and complex structure of torpedograss, thus reducing their foraging efficiency (Crowder & Cooper 1982).

### **Length Frequency Distributions**

The K-S tests for length frequency distributions of fishes were not significant with regards to effects of torpedograss stem density. Bluegill showed no significant effects of season or stem density on length frequencies. In contrast K-S tests for differences across seasons were significant for largemouth bass and western mosquitofish. More largemouth bass individuals were collected in the spring and summer than autumn and winter. Largemouth bass spawn in late winter or early spring, when water temperatures begin to rise (Coutant 1975). The length frequency distributions of largemouth bass show a significant increase in the size of individuals from winter to spring, and then from spring to summer. This could be due to offspring utilizing torpedograss as habitat, and remaining within it for multiple seasons.

More western mosquitofish were collected during the spring (n=41) than all other seasons combined (n=32). While western mosquitofish tend to spawn in warmer months, such as from March to October, photoperiod may affect reproduction initiation to a greater extent than water temperature (Lee and Burgess 1980, Davis 1978). The length frequency distributions of western mosquitofish show a significant increase in the size of individuals from spring to summer, and again from summer to fall. Similar to the largemouth bass, this may be due to offspring and adults utilizing torpedograss as habitat, and remaining within it for multiple seasons.

Significance tests may in part have been influenced by larger sample sizes for both largemouth bass and western mosquitofish as compared to bluegill and other fish species. Torpedograss is generally considered a wetland grass, or shoreline grass that does not usually grow in deeper water. Shallow water in all sample sites used in this study may have restricted the number of fishes that were present and available for sampling. In future studies, this potentially confounding factor might be addressed by increasing the number of sample sites, or extending sample site areas into deeper water further from shore.

### **Multivariate Fish Analysis**

There were significant effects observed for certain combinations of season, stem density, and vegetation weight, and the percentage of all variation that could be explained was 18.1%. Of the variation that could be explained, the exclusive effects of stem density and vegetation weight explained the greatest amount of variation (12.4%), accounting for over two thirds of all variation that could be explained by the ordination. This suggests that fishes may be utilizing the torpedograss as habitat, as previous studies show increased habitat complexity is associated with increased fish presence (Rennie et al. 2005, Savino et al. 1992, Becket et al. 1993).

The CCA of common fishes corroborates this data since small-bodied fishes such as western mosquitofish were much more strongly correlated to dense levels of torpedograss whereas largemouth bass were more strongly correlated to medium densities of torpedograss. Prior studies such as that conducted by Rodusky et al. (2013)

corroborate this, as over 90% of fishes found in densely vegetated torpedograss stands in lake Okeechobee, Florida, were western mosquitofish. In contrast to both largemouth bass and western mosquitofish, inland silversides and bullhead minnows tended to be negatively correlated to increasing levels of vegetation weight and categorical torpedograss density, being more strongly correlated with sparse densities and bare substrate sites.

### **Multivariate Invertebrate Analysis**

The RDA partitioning variation analysis for the invertebrates showed all combinations of interactions between the first group (season), second group (vegetation weight, stem density), and third group (location in water column), as well as unique variation for each of the three groups represented were significant ( $p = <0.002$ ). All of the invertebrates represented in the RDA, which only included invertebrates that explained 95% of the explained variation, were all negatively correlated with bare substrate, and benthic samples. Chironomid larvae, which were the prey item with the highest prey-specific abundance for bluegill, largemouth bass, and golden topminnows, tended to be very strongly correlated with sparse torpedograss sites, as well as with torpedograss stems. High numbers of chironomid larvae within the torpedograss is typical, as chironomid larvae are frequently one of the most abundant invertebrate species within freshwater systems (Cranston 1995, Epler 1995).

All other invertebrates other than chironomid larvae were closely correlated to increasing vegetation weight, as well as to medium and dense categories of torpedograss.



This correlation suggests that while increasing densities of torpedograss may not be best for foraging habitat for fishes due to decreasing sizes of interstitial spaces, the increasing densities of torpedograss are excellent habitat for the majority of invertebrates observed. Based on these observations, medium densities of torpedograss would be expected to be the ideal level of density to support fish assemblages and insectivorous or omnivorous fish foraging behavior.

### **Stomach Content Analysis**

Given the high stem densities of torpedograss, as well as abundance of invertebrates within torpedograss, invertivorous fish taxa would be expected to be more prevalent within torpedograss. This was shown to be true, as most of the ‘common fishes’, such as western mosquitofish, largemouth bass, golden topminnows, and bluegill were either insectivorous or omnivorous (Goldstein & Simon 1999). With regard to fish diets evaluated, chironomid larvae consistently had the highest prey-specific abundance value, followed closely by amphipods, across each species: largemouth bass ( $O_i=0.83$ ,  $P_i=49.89$ ), bluegill ( $O_i=1$ ,  $P_i=92.93$ ), and golden topminnows ( $O_i=0.03$ ,  $P_i=50$ ). No plant material was found in stomach contents for any of these three taxa, although both bluegill and golden topminnows are known to feed on vegetation as a large component of their diet (Etnier and Starnes 1993; Keast 1985). Thus, one could question whether or not these fishes are utilizing the torpedograss as a food resource, or just as a foraging location for associated invertebrate prey items.

## **Stable Isotope Analysis**

The stable isotope analysis supports the use of torpedograss as a foraging location, as the  $\delta^{13}\text{C}$  (‰) of animal tissues is highly dependent on the diet composition of the given animal (Camin et al. 2016). The  $\delta^{13}\text{C}$  (‰) values for the largemouth bass, bluegill, and western mosquitofish were all similar, and correlated to the  $\delta^{13}\text{C}$  (‰) value of the invertebrates and periphyton tested.  $\delta^{15}\text{N}$  (‰) is generally introduced to animal tissue through consumption of plant matter; and, the  $\delta^{15}\text{N}$  (‰) values within plants tend to be highly correlated to nitrates within the soils, which in turn are derived from atmospheric nitrogen (Camin et al. 2016). The  $\delta^{15}\text{N}$  (‰) value of torpedograss is relatively close only to that of the western mosquitofish, meaning that it is possible that the western mosquitofish at some point in their life could be consuming the torpedograss itself. It is more likely that the western mosquitofish is feeding on the periphyton living on the torpedograss, as the  $\delta^{13}\text{C}$  (‰) values for periphyton and the western mosquitofish are extremely similar.

A few changes could have been made to the sample design to improve accuracy of the results and assumptions presented. The overall sample size of the fish assemblage was fairly small, and only contained 208 individuals over all observed seasons. A larger sample size could have potentially rectified this issue; however, I believe it would be more efficient to instead increase the size of the sampled area within each site. As Lake Conroe has a relatively small slope, the depth of water one meter from the shoreline is relatively shallow, and is consistently less than 30 cm. As the samples taken for this study were 1x5 meters, increasing the sample area to 2x5 meters may have allowed for

greater numbers of fishes. Another issue could have been an underrepresentation of the western mosquitofish community. The blocknet that was utilized had 0.64-cm hexagonal mesh, and some western mosquitofish within the torpedograss were small enough that they could have escaped through the mesh. In future studies I would recommend a smaller mesh blocknet be used when electrofishing.

Overall, this study can give insight into the fish and invertebrate assemblages that are utilizing torpedograss as habitat and a potential foraging location. Identification of these metrics may be important, as this data suggests that torpedograss provides a utilizable habitat for both game and non-game fishes as well as providing habitat and a foraging location for many species of invertebrates. As such, these findings have the potential to assist fisheries biologists and land managers when planning future fisheries management actions that may influence biota utilizing torpedograss. Pearson and Ortega (2009) suggested a complete eradication invasive species such as torpedograss in aquatic systems, which can be not only incredibly costly, but may also be unnecessary. Instead, management of torpedograss may be preferable as it would reduce costs while still providing habitat and a foraging location to both fishes and invertebrates.

## REFERENCES

- Amundsen, P. A., H. M. Gabler, and F. J. Staldvik. 1996. A new approach to graphical analysis of feeding strategy from stomach contents data- modification of the Costello method. *Journal of Fish Biology* 48:607-614.
- Anderson, R. O. and R. M. Neumann. 1996. Length, weight, and associated structural indices. Pages 447-482 in B. R. Murphy and D. W. Willis, editors. *Fisheries Techniques*, 2nd edition. American Fisheries Society, Bethesda, Maryland.
- Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Invertebrates and Fish*, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.
- Beckett, D.C., T.P. Aartila, and A. Miller. 1992. Contrasts in density of benthic invertebrates between macrophyte beds and open littoral patches in Eau Galle Lake, Wisconsin. *Am.Midl. Nat.* 127: 77-90.
- Bettoli, P.W. and J. E. Morris. 1991. Changes in the abundance of two atherinid species after aquatic vegetation removal. *Transactions of the American Fisheries Society* 120:90-97
- Bodin, N., F. Le Loc'h and C. Hily. 2007. Effect of lipid removal on carbon and nitrogen stable isotope ratios in crustacean tissues. *Journal of Experimental Marine Biology & Ecology*, 341:168-175
- Camin, F., L. Bontempo, M. Perini, and E. Piasentier, 2016. Stable Isotope Ratio Analysis for Assessing the Authenticity of Food of Animal Origin. *Comprehensive Reviews in Food Science & Food Safety*, 15: 868-877. doi:10.1111/1541-4337.12219
- Conrow, R., A. V. Zale, and R. W. Gregory. 1990. Distributions and abundances of early life stages of fishes in a Florida lake dominated by aquatic macrophytes. *Transactions of the American Fisheries Society* 119:521-528
- Costello, M. J. 1990. Predator feeding strategy and prey importance: a new graphical analysis. *Journal of Fish Biology* 36:261-263.
- Coutant, C.C. 1975. Responses of bass to natural and artificial temperature regimes, pp. 272-285. In: *Black bass biology and management*. H. Clepper, ed. Sport Fishing Institute, Washington, D.C. 534 pp.

- Cranston, P.S. 1995. Introduction. In Armitage, P.D.; P.S. Cranston; and L.C.V. Pinder eds. *The Chironomidae: the Biology and Ecology of Non-biting Midges*. pp 1-7
- Crowder, L. B. and W. E. Cooper. 1979. Structural complexity and fish prey interaction in ponds: a point of view. Pages 345-461 *in* D. L. Johnson and R. A. Stein, editors. *Response of fish to habitat structure in standing water* North Central Division, American Fisheries Society, Special Publication 6.
- Crowder, L. B., and W. E. Cooper. 1982. Habitat structural complexity and the interaction between bluegills and their prey. *Ecology* 63(6):1802-1813.
- Davis, J.R. 1978. Reproductive seasons in *Gambusia affinis* and *Gambusia geiseri* (Osteichthyes: Poeciliidae) from southcentral Texas. *Texas J. Sci.* 30(1):97-99.
- DeNiro, M. J., and S. Epstein. 1978. Influence of diet on the distribution of carbon isotope in animals. *Geochim. Cosmochim. Acta.*, 42: 495.
- Dibble, E. D., J. K. Kilgore, and S. L. Harrel. 1996. Assessment of fish-plant interactions. *American Fisheries Society Symposium* 16:357-372.
- Durocher, P. P., W. C. Provine, and J. E. Kraai. 1984. Relationship between abundance of largemouth bass and submerged vegetation in Texas reservoirs. *North American Journal of Fisheries Management* 4:84-88.
- Epler, J.H. 1995. Identification Manual for the Larval Chironomidae (Diptera) of Florida Revised Edition. Bureau of Surface Water Management, Florida Department of Environmental Protection
- Etnier, D. A., W.C. Starnes. 1993. *The Fishes of Tennessee*. 363.
- Freckmann, R. W., and M. G. LeLong. 2006. *Panicum hemitomon*. Manual of the Grasses for the United States, Utah State University.  
<http://www.herbarium.usu.edu/webmanual/>. Accessed 30 September 2015.
- Fry, B. 2006. *Stable Isotope Ecology*. New York: Springer. Print.
- Goldstein, R.M., and T.P. Simon. 1999. Toward a united definition of guild structure for feeding ecology of North American freshwater fishes. pp. 123-202 *in* T.P. Simon, editor. *Assessing the sustainability and biological integrity of water resources using fish communities*. CRC Press, Boca Raton, Florida. 671 pp.
- Hanlon, C.G., and K. Langeland. 2000. Comparison of experimental strategies to control torpedograss. *Journal of Aquatic Plant Management* 38:40-47

- Havens, K.E., and D.E. Gawlik. 2005. Wetlands 25: 908. doi:10.1672/0277-5212(2005)025[0908:LOCEM]2.0.CO;2
- Holm, L. G., D. L. Plucknett, J. V. Pancho, and J. P. Herberger. 1977. The World's Worst Weeds: Distribution and Biology. University Press of Hawaii, Honolulu.
- Hoyer, M. V., and D. E. Canfield, Jr. 1996. Largemouth bass abundance and aquatic vegetation in Florida lakes: An empirical analysis. *Journal of Aquatic Plant Management* 34: 23-32
- Jackson, D. A., P. R. Peres-Neto, and J. D. Olden. 2001. What controls who is where in freshwater fish communities - the roles of biotic, abiotic, and spatial factors. *Canadian Journal Aquatic Science* 58:157-170.
- Keast, A. 1984. The introduced aquatic macrophyte, *Myriophyllum spicatum*, as habitat for fish and their invertebrate prey. *Canadian Journal of Zoology* 62:1289-1303.
- Keast, A. 1985. Planktivory in a littoral-dwelling lake fish association: prey selection and seasonality. *Canadian Journal of Fisheries and Aquatic Sciences* 42:1114-1126.
- Kiljunen, M., J. Grey, T. Sinisalo, C. Harrod, H. Immonen, and R. I. Jones. 2006. A revised model for lipid-normalizing  $\delta^{13}\text{C}$  values from aquatic organisms, with implications for isotope mixing models. *Journal of Applied Ecology*, 43, 1213.
- Kirkman, L. K., and R. R. Sharitz. 1993. Growth in controlled water regimes of three grasses common in freshwater wetlands of the southeastern USA. *Aquatic Botany*, 44:345-359.
- Lee, D.S., and G.H. Burgess. 1980. *Gambusia affinis* (Baird and Girard), Mosquitofish. pp. 538 in D. S. Lee, et al. *Atlas of North American Freshwater Fishes*. N. C. State Mus. Nat. Hist., Raleigh, i-r+854 pp.
- Linam, G.W., L.J. Kleinsasser, and K.B. Mayes. Regionalization of the Index of Biotic Integrity for Texas Streams. Rep. Texas Parks and Wildlife Department, n.d. Web. 11 Oct. 2016. <<https://repositories.lib.utexas.edu/handle/2152/6715>>.
- Merritt, R. W., K. W. Cummins, and V. H. Resh. 1984. Collecting, sampling, and rearing methods for aquatic insects. Pages 11-26 in R. W. Merritt and K. W. Cummins (editors). *An introduction to the aquatic insects of North America*. Kendall/Hunt, Dubuque, Iowa.
- Merritt, R.W., K.W. Cummings, editors. 1996. *An introduction to the aquatic insects of North America*. 3rd edition. Kendall / Hunt. Dubuque, Iowa.

- Mitsch, W.J., R.H. Mitsch, R.E. Turner. 1994. Wetlands of the Old and New Worlds ecology and Management. In: Mitsch WJ (ed) Global wetlands: old world and New Amsterdam. Elsevier, Amsterdam, pp 3–56
- Neuman, R. M. and M. S. Allen. 2007. Size Structure. Pages 375 - 422 in C. S. Guy and M. L. Brown, editors. Analysis and interpretation of freshwater fisheries data. American Fisheries Society, Bethesda, Maryland.
- Pearson, D.E. 2009. Invasive plant architecture alters trophic interactions by changing predator abundance and behavior. *Oecologia* 159:549–558
- Pearson, D.E., and Y. Ortega. 2009. Managing invasive plants in natural areas: Moving beyond weed control. In: Kingely RV (ed) Weeds: management, economic impacts and biology. Nova Science, New York
- Pinnegar, J. K, and V.C. Polunin. 1999. Differential fractionation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  among fish tissues: implications for the study of trophic interactions. *Functional Ecology*. 13, 225.
- Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, 83, 703.
- Post, D. M., C. A. Layman, D. A. Arrington, G. Takimoto, J. Quttrochi, and C. G. Montana. 2007. Getting to the fat of the matter: models, methods, and assumptions for dealing with lipids in stable isotope analyses. *Oecologia*, 152, 179.
- Rennie, M. D., and L.J. Jackson. 2005. The influence of habitat complexity on littoral invertebrate distributions: patterns differ in shallow prairie lakes with and without fish. *Canadian Journal of Fisheries & Aquatic Sciences*, 62(9), 2088
2099. doi:10.1139/F05-123
- Reynolds, J. B. 1996. Electrofishing. Pages 147-163 in B. R. Murphy, and D. W. Willis, editors. Fisheries techniques 2nd Edition. American Fisheries Society, Bethesda, MD.
- Rodusky, A.J., B. Sharfstein, C.G. Hanlon et al. 2013. Wetlands Ecology and Management. 21: 87. doi:10.1007/s11273-013-9281-y
- Savino, J., et al. 1992. "Bluegill growth as modified by plant density: an exploration of underlying mechanisms." *Oecologia* 89(2): 153-160.

- Spotte, S. 2007. Bluegills: biology and behavior. American Fisheries Society, Bethesda, Maryland.
- Sutton, D. L. 1996. Growth of torpedograss from rhizomes planted under flooded conditions. *Journal of Aquatic Plant Management*. 34: 50-53.
- Tarver, D. P. 1979. Torpedograss (*Panicum repens* L.). *Aquatics* 1: 5-6.
- ter Braak, C.F. and P. Smilauer. 2002. CANOCO Reference manual and CanoDraw for Windows user's guide: Software for Canonical Community Ordination (version 4.5). Microcomputer Power, Ithaca, NY
- Warren, G.L., and D.A. Hohlt. 1994. Lake Okeechobee community invertebrate investigations. Study IV in the Lake Okeechobee- Kissimmee River-Everglades Resource Evaluation Report. Wallop-Breaux Project F-52 Completion Report to the United States Department of Interior, Washington
- Waterhouse, D. F. 1994. Biological Control of Weeds: Southeast Asian Prospects. Australian Centre for International Agricultural Research, Canberra, Australia.
- Wege, G. J., and R. O. Anderson. 1979. Influence of artificial structure on largemouth bass and bluegills in small ponds. Pages 592-69 in R. A. Stein and D. L. Johnson, editors. Response of fish to habitat structure in standing water. American Fisheries Society, North Central Division, Special Publication 6, Bethesda, Maryland.
- Zelder, J.B., and S. Kercher. 2004. Causes, consequences of invasive plants in wetlands: opportunities, opportunists and outcomes. *Critical Reviews in Plant Science* 23(5):431-452